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Genetic variation in natural populations of Daphnia .

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GENETIC VARIATION IN NATURAL POPULATIONS OF *DAPHNIA*

by

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To my husband, Gary, and my family.

ABSTRACT

Polyacrylamide electrophoresis was used to study allozyme variation at 16 loci in ten natural populations of the cyclic parthenogen, Daphnia magna (Cladocera: Crustacea) from Churchill, Manitoba. These populations possessed extremely low levels of variability; polymorphism was found at only one locus in two of the populations. The frequency of the rare allele was 0.03 in one populations and 0.02 in the other. All ten populations were monomorphic for the same allele at the other 15 loci.

This data was compared to earlier work done by Hebert (1972) on 25 populations of D. magna from Cambridge, England. These populations possessed considerably more variation than Arctic ones. Average heterozygosity per individual, based on 11 loci, was 0.07. Variation was found at three of the 11 loci. Nei's statistics I, genetic similarity, and D, genetic distance, were used to measure the amount of genetic divergence between the English and Arctic groups of populations. Within-group similarity was high, pairs of Arctic populations had an average similarity of 1.00 while average similarity for English pairs was 0.946. In contrast, inter-group comparisons showed that extensive genetic differentiation had occurred between the two metapopulations. Average similarity between English-Arctic pairs was 0.62. Analysis of laboratory clones from one

English and two Arctic populations revealed gene substitutions at 6 out of 16 or 38% of the loci examined. This is the lowest genetic similarity recorded to date between two populations of the same species.

Hybridization studies were carried out to determine if individuals from the two strains where capable of interbreeding. Thirty-eight hybrid offspring from crosses between English males and Arctic females were hatched and maintained in culture until they reached maturity. All of them were viable and fertile.

Competition experiments were undertaken to compare the fitness of the hybrids with their parent clones. Six hybrids were competed with each parent separately at two temperatures. One clone was superior to both of its parents at both temperatures while one was inferior in all situations. The others showed variable competitive ability depending on the temperature and the parent with which it was competing. The hypothesis that outcrossing to genetically distinct clones may be advantageous for generating new genotypes within natural Daphnia populations is discussed.

Many experiments with D. magna required the use of sexual females and males. Experiments were undertaken to determine what set of environmental conditions successfully induces sexual reproduction. Arctic clones were extremely sensitive to short-day photoperiods, producing many males and ephippial females. English clones were less sensitive to photoperiod. They responded optimally to crowding and a

rapid decline in the quality of their environment. These results were discussed in relation to the differences between the habitats of the two strains.

It was also discovered that Arctic females released unfertilized eggs into their ephippia in the absence of males. Tetrazolium staining indicated that about 30% of these unfertilized eggs might be viable but attempts at hatching them were unsuccessful. This leads to the possibility of facultative sex in the Arctic strain of D. magna. This may be related to the short growing season of the Arctic environment where it is necessary to produce resting eggs every year even if males are scarce.

Several zooplankton biologists have noted a correlation between swarming and the presence of ephippial females and males. This has led to the suggestion that one sex is able to attract the other. Experiments designed to determine if ephippial females and males released sex phermones to attract one another were undertaken. The results strongly suggested that no such substances were produced. The production of such chemicals seem unnecessary since sex usually occurs during periods of high density when the chances of finding a mate are good.

Allozyme variation at 11 loci was studied in 11 populations of another cyclic parthenogen, D. pulex, from southwestern Ontario. Results showed that all of these populations were, in fact, reproducing by obligate parthenogenesis. Marked deviations from Hardy-Weinberg equilibrium were ob-

served at all polymorphic loci. Analysis of laboratory clones from each population revealed many inter-locus associations between genotypes. Altogether 22 different clones were recognized with one to seven being found in a single habitat. Coexistance of clones contradicts the principle of competitive exclusion. A mechanism for the coexistance of clones, involving temporal variation in clonal fitnesses, is discussed.

ACKNOWLEDGEMENTS

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CHAPTER I

GENERAL INTRODUCTION

CHAPTER I

The process of evolution by natural selection requires the existance of genetic variation. Therefore, in order to understand the mechanisms by which evolution occurs it is first necessary to know how much genetic variation actually exists in natural populations. Until recently variation could only be measured on the basis of visible characteristics. Problems arose because an organism's phenotype is the result of complex interactions between the genotype and the environment. In most cases there is no method for correlating the visible phenotype with the underlying genotype producing it. In 1966 Lewontin and Hubby used the technique of starch gel electrophoresis to study genetic variation in the fruit fly Drosophila pseudoobscura. This technique allowed them to look at variation in the products of individual loci and thus the phenotype can be directly related to the genotype for a more accurate estimate of variability. Since then many geneticists have used this technique in an effort to measure the amount of genetic variation in natural populations. Table 1.1 shows a brief summary of some of the results that have been obtained. In general invertebrates seem to possess somewhat more variability than vertebrates. The mean proportion of polymorphic loci in invertebrates was 0.47 compared to 0.25 in vertebrates. Similarly the mean heterozygosity per individual in invertebrates was 0.135 while it was only 0.061 in vertebrates.

Table 1.1 Comparison of Genetic Variation in Animals (Hamrick,1979)

Group	Number of Species or Forms	Mean Number of Loci per Species	Mean Proportion of Loci Polymorphic per Population (P) (SD)	Mean Proportion of Loci Heterozygous per Individual (H) (SD)
Insects				
Drosophila	28	24	0.529(.030)	0.150(.010)
Others	4	18	0.531	0.151
Haplodiploid wasps	6	15	0.243(.039)	0.062(.007)
Marine invertebrates	9	26	0.587(.084)	0.147(.019)
Snails				
Land	5	18	0.437	0.150
Marine	5	17	0.175	0.083
Total Invertebrates	57	Mean=21.8	Mean=0.469	Mean=0.135
Fish	14	21	0.306(.047)	0.078(.012)
Amphibians	11	22	0.336(.034)	0.082(.008)
Retiles	9	21	0.231(.032)	0.047(.008)
Birds	4	19	0.145	0.042
Rodents	26	26	0.202(.015)	0.043(.005)

Table 1.1 Continued

Group	Number of Species or Forms	Mean Number of Loci per Species	Mean Proportion of Loci Polymorphic per Population (P) (SD)	Heterozygous per Individual (H) (SD)
Large mammals	4	40	0.233	0.037
Total Vertebrates		Mean=$\overline{24}$.1	Mean=$\overline{0.247}$	Mean=$\overline{0.061}$

Most studies have shown that animals possess considerably more genetic variation than was once thought. This has led to the formation of many hypotheses concerning the maintenance of so much variation. Of primary concern are the forces acting on it such as recombination, selection and genetic drift.

Until recently, most of the work has concerned sexually reproducing organisms. However, geneticists have now realized that the study of organisms with unusual breeding systems may provide new insights into the forces creating and maintaining genetic variation. One group of organisms with an unusual mode of reproduction is the species of the genus Daphnia (Cladocera). These planktonic microcrustaceans are found in fresh-water lakes and ponds throughout the world from the tropics to the arctic. Several features of these organisms (aside from their mode of reproduction) make them excellent for genetic studies. As mentioned above, they have wide geographic ranges making them especially suitable for macrogeographical studies. Furthermore, populations are well defined and consist of large numbers of individuals. Daphnia are major components of many fresh-water plankton communities and consequently much is known of their general biology and ecology. In addition many species are easy to culture in the laboratory.

Daphnia species reproduce either by cyclic or obligate

parthenogenesis, the former being more common. Populations of cyclic parthenogens normally consist entirely of females produced by apomicitic thelytoky. During the formation of eggs only one division, a maturation division, occurs (Zaffagnini and Sabelli, 1972) resulting in offspring that are genetically identical to their mothers (Hebert and Ward, 1972). The eggs are released into the females' brood pouch where they complete development. When the offspring are released they resemble miniature versions of the adult. Growth proceeds through a series of moults or instars. Juveniles usually go through four or five instars before reaching reproductive maturity. Young are released prior to each adult moult and a new batch of eggs is deposited into the brood pouch immediately afterwards.

Under certain environmental conditions females switch to sexual reproduction, producing males and haploid eggs. Males are produced from parthenogenetic eggs and are thus genetically identical to their mothers. Crowding seems to be an important stimulus for male production. The critical period for sex determination seems to be the three to four hours before the beginning of cleavage (Banta and Brown, 1929). Ruvinsky et al., (1978) suggested that the appropriate environmental stimuli activate the program of genes in the egg which are responsible for maleness while the genes for femaleness are inactivated.

Haploid eggs are produced normally by meiosis and re-

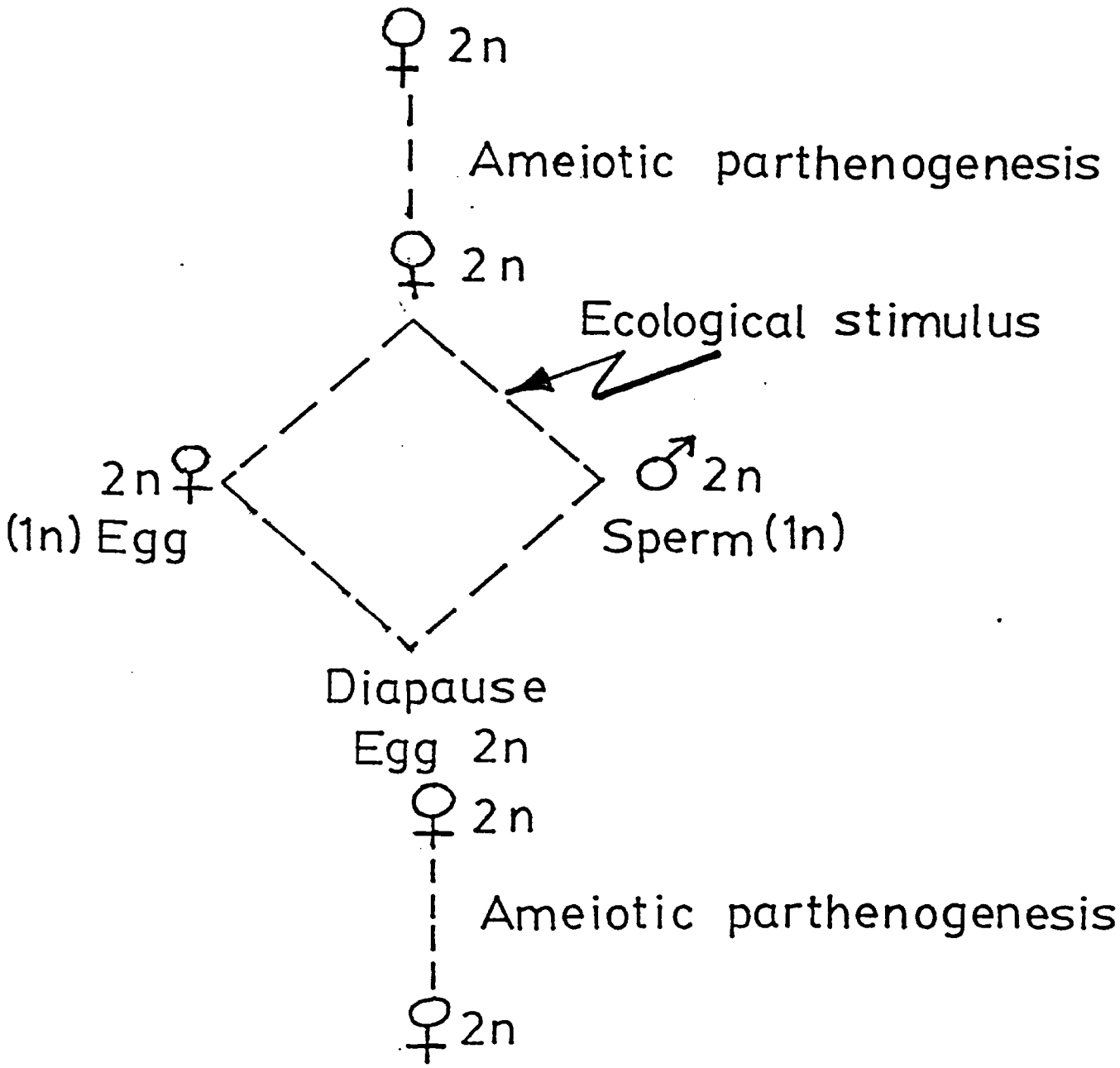
quire fertilization by the males' sperm. Several factors have been implicated in the production of sexual eggs, for example, decreasing photoperiod, crowding, decreasing temperature and decreasing food levels (Stross and Hill, 1968; Stross, 1969; Stimpfl, 1971; Slobodkin, 1954).

Sexual eggs are enclosed in a structure called an ephippium which is formed from many layers of hardened carapace deposited around the brood pouch. Ephippial eggs are resistant to freezing, dessication and digestive enzymes and they normally undergo a period of diapause before development. All ephippial eggs hatch into females. The life cycle of a cyclic parthenogen is diagrammed in Figure 1.1 (Ruvinsky et al., 1978). The life cycle of an obligate parthenogen is very similar except that the ephippial eggs are produced ameiotically as well.

Ephippia are responsible for re-establishing Daphnia populations in lakes and ponds that dry up or freeze during part of the year. Sexual reproduction occurs regularly in these intermittent populations while it may not occur for many generations or years in permanent bodies of water. In addition, ephippia are the sole means of dispersal between habitats; being carried by the wind or flood waters or on the feet or in the digestive tracts of animals and aquatic birds.

The genetic consequences of cyclic parthenogenesis are likely to be quite different from sexual reproduction. Young (1979b) has outlined several characteristics of Daph-

Figure 1.1 Life Cycle of a Cyclic Parthenogen

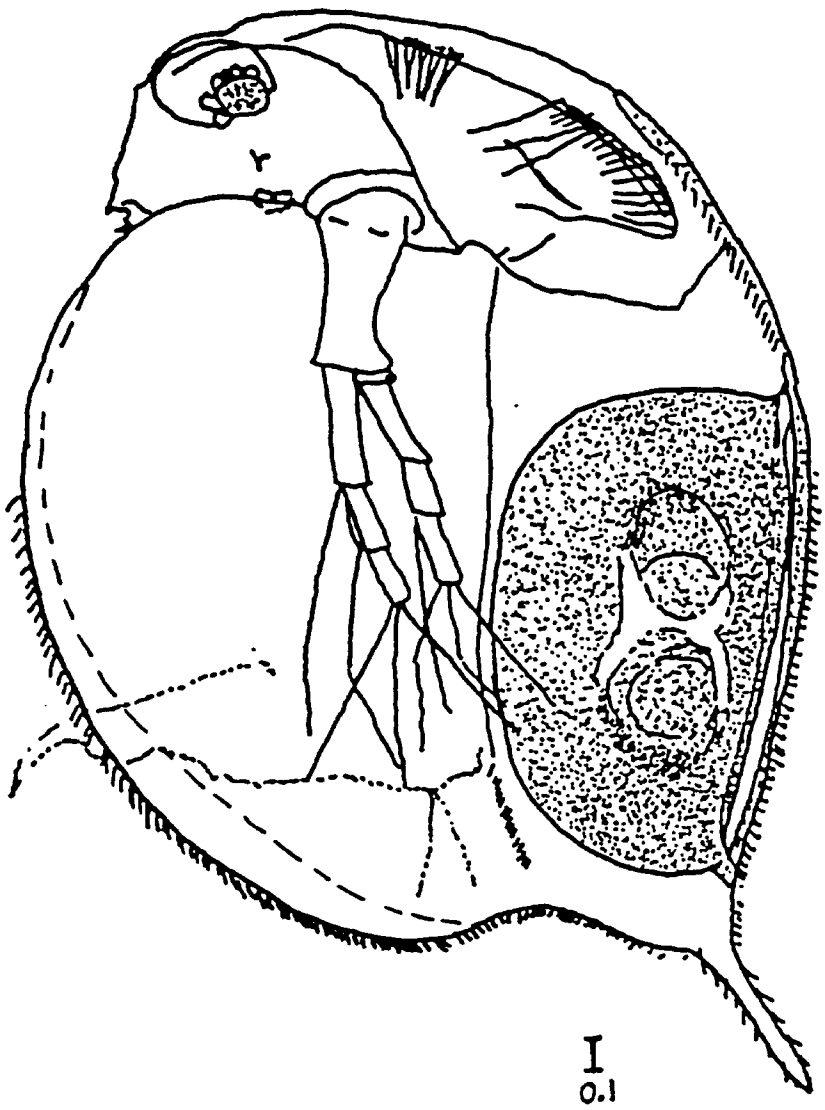


nia as compared to sexual organisms. The effective population size for sexual reproduction in permanent Daphnia populations is quite small, probably even less than the number of genetically distinct clones due to differences in clonal abundance and timing of sex . If clones became sexual at different times the frequency of selfing would be increased. In addition selection coefficients per generation might be very large in all types of populations. Fitness differences will be magnified during clonal reproduction; a slight disadvantage in each parthenogenetic generation could be effectively lethal over a long period of time. Finally, mutation rates may be higher due to the increased number of mitoses between each meiosis. If one assumes that mutation rates per parthenogenetic generation are the same as in a normal sexual generation, recessive deleterious alleles would tend to accumulate causing an increased mutational load at each meiosis.

Understanding the genetic characteristics of organisms such as Daphnia and how they are affected by selection, mutation and inbreeding should help in our understanding of the effects of these forces in populations of purely sexual organisms.

The main purpose of the work described here is to analyse the pattern of genetic variation in populations of Daphnia from various habitats using the technique of gel electrophoresis. In 1972 Hebert undertook a study of genetic structure in populations of D. magna (Figure 1.2)

Figure 1.2 Diagram of Ephippial *D. magna* Female



from Brooks, 1957

from England using this technique. He found much genetic heterogeneity among these populations including many cases of Hardy-Weinberg deviation along with significant gene frequency changes with time. This particular species is found in North America as well as in Europe so a study of several populations from northern Canada was undertaken (Chapter III). This provided an excellent opportunity to study variation on a macrogeographic scale. Because of the magnitude of genetic differentiation that was found between the English and Canadian D. magna, hybridization studies were performed to look for evidence of reproductive isolation and/or heterosis (Chapter IV).

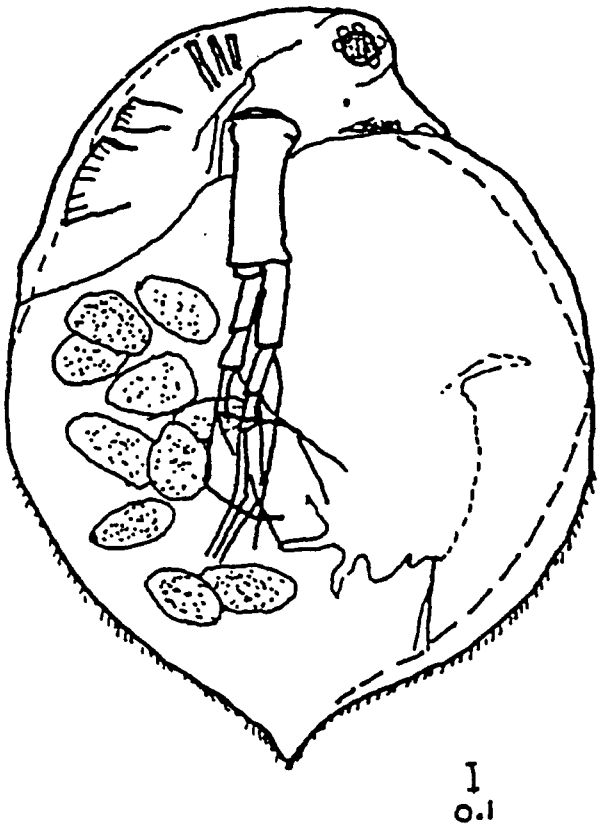
During the course of these investigations it was necessary to do breeding experiments which, of course, require the use of ephippial females and males. Considerable work has been done on ephippia and male induction in D. pulex (Stross and Hill, 1968; Stross, 1969; Stimpfl, 1972) but similar studies with D. magna have met with little success (Bunner and Halcrow, 1977). As a result, experiments concerning the production of ephippial female and male D. magna were attempted in order to determine which factors were most important in the initiation of sexual reproduction in this species (Chapter V).

Most studies of Daphnia both in the laboratory and in the field involve parthenogenetic females and therefore little is known of mating behaviour in this organism. As a result, several experiments concerning this aspect of

their behabiour were also performed (Chapter VI).

Of the numerous species of Daphnia that have Holarctic distributions, one of considerable interest is D. pulex (Figure 1.3), which is also thought to be a cyclic parthenogen. This species is found in small intermittent or permanent ponds as well as in larger permanent lakes. In order to determine how the population structure of D. magna compared to other species, a study of genetic variation in several populations of D. pulex from southwestern Ontario was undertaken (Chapter III).

Figure 1.3 Diagram of D.pulex Female



from Brooks, 1957

CHAPTER II

MATERIALS AND METHODS

CHAPTER II

COLLECTION OF SAMPLES

Daphnia were collected from ponds using a plankton net. Depending on the depth and diameter of the pond, the net was either thrown out from shore or the collector waded into the pond pulling the net through the water. If the pond was fairly deep care was taken to make several throws of the net, to sample near the surface of the water as well as near the bottom of the pond. In addition, large ponds were sampled from several sites along their perimeters.

The *Daphnia pulex* populations from southwestern Ontario were sampled with a 64μ net which was thrown from shore. The Churchill, Manitoba ponds were sampled by wading through the ponds with a 500μ net. These ponds were often quite shallow and thus the net could not be thrown without its sinking into the mud. A few of the ponds were very small being little more than a puddle of water formed among some rocks. In such cases the net was merely dipped into the water from the edge.

The animals were put into capped, two liter plastic bottles filled with pond water. Very few animals died when transported short distances in these bottles. The animals from Churchill, Manitoba had to be shipped to Win-

dsor, Ontario. Just before shipping, oxygen was added to each bottle and then they were placed in a cooler containing ice packs. This method worked very well as very few animals died during the two day trip to Windsor.

Animals were stored alive at 5°C or frozen in distilled water for use in electrophoretic studies. Some animals were isolated and used to start laboratory cultures.

CULTURE TECHNIQUES

During the early part of this work Daphnia were cultured in water from a pond in Sandwich West Township near Windsor. This pond did not dry up in the summer, having water in it even during August. The water was kept in 20 liter carboys and recycled for several months. Needless to say enough water had to be collected in October to last until spring. The animals did very well in fresh pond water but as it aged the condition of the animals began to deteriorate. Transferring them to fresh water caused immediate improvement of unhealthy cultures.

Collecting large amounts of pond water was inconvenient as was the fact that fresh water was unavailable during the winter. Furthermore, the exact composition was unknown making it unsuitable for more carefully controlled experiments. As a result it was decided to find an artificial medium that the animals could be grown in. This medium (Dr. R.W. Winner, pers. comm.) proved to be very successful when used in conjunction with appropriate food. The medium consisted of;

0.96 grams $NaHCO_3$

0.76 grams $CaSO_4.2H_2O$

0.6 grams $MgSO_4$

0.01 grams KCl

20 liters distilled H_2O

Several different types of food were tried with variable success. Initially the animals were fed a suspension of baker's yeast. This proved to be unacceptable as the condition of the animals varied greatly. If too little yeast was fed the animals would run out of food between feedings. On the other hand too much yeast had a detrimental effect, being particularly harmful to neonates.

A mixture of yeast and laboratory cultured Euglena was also tried. The Euglena cultures became contaminated with bacteria very easily so this organism was abandoned as a food source.

Several people have tried using various algal species such as Chlorella, Chlamydomonas and Ankistrodesmus as food for Daphnia. Because of the ease with which it is cultured we tried Chlamydomonas (Strain 86 W 0100)from Ward's Natural Science Estab., Inc.). Stock cultures were maintained on BG-11 slants (Table 2.1). Large cultures were grown in two liter Pyrex flasks in Bold's Basic Medium under continuous light from 40 W "Cool White" fluorescent tubes (Table 2.2). The cultures were used after two weeks of growth. The optical density at 620 nm was 0.15.

Chlamydomonas alone did not prove to be an adequate food. As a result we supplemented the algae with a vitamin solution (R.W. Winner, pers. comm.) and a 2 mg/ml suspension of liver (Difco Bactoliver) (Murphy and Davidoff, 1972) in the following proportions; six parts Chlamydomonas,

Table 2.1 Composition of BG-11 Agar Slants* Used in Maintaining *Chlamydomonas* Cultures

Basal salts (grams/liter H_2O)	
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.07
Na_2CO_3	0.02
$CaCl_2.2H_2O$	0.02
$Na_2SiO_3.9H_2O$	0.05
EDTA	0.001
Citric acid	0.006
Iron citrate	0.006

To this add 1.0 ml/liter of separately sterilized trace salts solution:

	(grams/liter)
H_3BO_4	2.9
$MnCl_2.4H_2O$	1.8
$ZnSO_4.7H_2O$	0.22
$Na_2MoO_4.2H_2O$	0.4
$CuSO_4.5H_2O$	0.08
$Co(NO_3)_2.6H_2O$	0.05

For solid medium use 1% agar.

* From Biology Department, University of Windsor.

Table 2.2 Liquid Medium Used in Culturing *Chlamydomonas*.

Stock Solutions		
1.	$NaNO_3$	25 grams
	$MgSO_4.7H_2O$	5 grams
	H_2O	1 liter
2.	K_2HPO_4	10 grams
	KH_2PO_4	15 grams
	NaCl	2.5 grams
	H_2O	1 liter
3.	$CaCl_2.2H_2O$	3.3 grams
	H_2O	1 liter
4.	Soil Extract	
	1 volume	soil
	2 volumes	water
	Autoclave for 30 minutes	
	Dilute supernatant approximately by half.	

Algal Medium

1 liter H_2O

10 mls	Solution 1	
10 mls	Solution 2	
10 mls	Solution 3	Autoclave separately
50 mls	Soil extract	

one part liver suspension and two parts vitamin solution. The vitamin solution was made by adding one ml of a vitamin concentrate to 19 mls of water. It was added to the food once per week. The composition of this concentrate is shown in Table 2.3.

This combination of food and artificial culture medium proved to be very successful for maintaining healthy Daphnia cultures in the laboratory. One problem, however, was growing enough algae to feed all the cultures. Dr. Robert Peters (pers. comm.) suggested a goldfish aquarium as possible source of algae. The primary species of algae that grows in the aquarium is Scenedesmus however numerous other algal species and protozoans were also present. A mixture of 120 mls liver suspension and 40 mls of vitamin concentrate (once per week) in two liters of algae was used to feed the Daphnia.

Cultures were kept either in 1½ liter glass jars or 100 ml plastic beakers. One hundred and fifty mls of the Scenedesmus/liver mixture were added to the jars twice each week while four mls were added to beakers three times per week. Stock cultures were kept at room temperature in continuous light from the fluorescent fixtures in the room. Any modifications of this procedure pertaining to individual experiments will be discussed later along with the particular experiments themselves.

Table 2.3 Vitamin Solution Concentrate

Ca Pantothenate	700	mgrams
B_{12}	0.08	mgrams
Thiamin	60	mgrams
Riboflavin	40	mgrams
Nicotinamide	130	mgrams
Folic acid	330	mgrams
Choline	500	mgrams
Putrescine	30	mgrams

ELECTROPHORESIS

Allozyme studies were carried out using discontinuous polyacrylamide gel electrophoresis. Two gel buffer systems and two gel concentrations were employed to allow the analysis of 15 enzyme systems. The procedures used are those of Hebert and Ward (1972) and Hebert (pers. comm.). Further details of staining procedures can be found in Harris and Hopkinson (1976).

Gel Production

Approximately 100 glass tubes (5 mm x 75 mm) were placed in a plexiglass cylinder (inside diameter 80 mm) that had been sealed at one end with a rubber stopper. Two hundred and forty mls of gel solution were poured into the tubes which were then overlayered with distilled water. Gels were allowed to set overnight and were used the next day. Twotypes of gels, Tris-Borate and Tris-HCl,were used. The solutions used to make the gels are described below. All chemicals are from Sigma Chemical Company unless otherwise stated.

Gel Buffers

Tris-HCl Solution X

190 mls H_2O
366 g Tris
480 mls 1N HCl
2.3 mls TEMED
H_2O to total 1 liter

Tris-Borate Solution X

25 mls .5M Boric Acid
80 mls .25M Tris
500 mls H_2O
1.75 mls TEMED

Acrylamide Solution Y

140 grams Acrylamide

3.675 grams N'N'-Methylene Diacrylamide (Bis)(BDH Chemicals)

500 mls H_2O

To Make 7.5% Gels:

Tris-HCl	1 part X 2 parts Y 3 parts H_2O
Tris-Borate	1 part X 1 part Y 2 parts H_2O

Add Ammonium Persulphate according to Table 2.4

To Make 10% Tris-HCl Gels:

Use 4/3 the normal amount of Y and reduce water by an amount equal to 1/4 the amount of Y added. Use the normal amount of X.

Procedure

Single animals were crushed on a plexiglass plate in a drop of distilled water. The suspension was absorbed onto a 5 mm disc of Whatman #1 filter paper which was then placed on the surface of a gel. The gel tubes were put into the electrophoresis tank and a solution of bromphenol blue in .25M sucrose was applied to the surface of each gel. The electrode buffer used was Tris-Glycine pH 8.3. A concentrate consisting of 120 grams Tris and 576 grams glycine in a total volume of 20 liters distilled water was

diluted 1 in 10 to make this buffer. Tris-Borate gels were run at approximately 1 mA per tube while Tris-HCL gels required 1 mA per tube for 10 minutes and then 3 mA per tube thereafter. Electrophoresis was continued until the bromphenol blue band was about 1 cm from the bottom of the gel tube. The gels were removed from the tubes and stained in the dark at 37°C. The type of gel used depended on the enzyme being studied. A description of the conditions required for each enzyme is given in Table 2.4.

Staining

All enzyme stains required one of the following buffers:

Tris-Maleate pH 5.3

48.4 grams Tris
46.4 grams Maleic acid
96 mls 1N NaOH
H_2O to a total of 4 liters

Tris-HCl pH 7.0

44.4 grams Tris
350 mls 1N HCl
H_2O to a total of 4 liters

Tris-HCl pH 9.0

96.8 grams
120 mls
H_2O to a total of 4 liters

The individual stains for each enzyme are described below.

NAD Nicotinamide Adenine Dinucleotide

NADP Nicotinamide Adenine Dinucleotide Phosphate

INT p-Iodonitrotetrazolium Violet

PMS Phenazine Methosulphate

Lactate dehydrogenase 1.1.1.27

Tris-HCl pH 9.0	1 ml
Na Lactate	0.1 ml
NAD	1 mg
INT	0,25 mg
PMS	trace

Malate dehydrogenase 1.1.1.37

H_2O	1 ml
Na Malate Solution	0.2 mls
NAD	1 mg
INT	0.25 mg
PMS	trace

Na Malate Solution

Tris-HCL pH 9	20 mls
Malate	3.68 g
H_2O	180 mls

Xanthine dehydrogenase 1.2.1.37

Tris-HCl pH 7.0	1 ml
Hypoxanthine	1 mg
NAD	1 mg
INT	0.25 mg
PMS	trace

Glucose-6-phosphate dehydrogenase 1.1.1.49

Tris-HCl pH 9.0	1 ml
Na Glucose-6-phosphate	0.5 g
NADP	0.2 mg
INT	0.25 mg
PMS	trace

Fumarase 4.2.1.2

Tris-HCl pH 9.0	1 ml
Na Fumarate	6 mg
Malic dehydrogenase	0.003 mls
NAD	1 mg
INT	0.25 mg
PMS	trace

Phosphoglucoisomerase 5.3.1.9

Tris-HCl Ph 7.0	1 ml
Fructose-6-phosphate	0.4 mg
Glucose-6-phosphate dehydrogenase	0.004 ml (400 U/ml)
NADP	0.2 mg
INT	0.25 mg
PMS	trace

Phosphoglucomutase 2.7.5.1

Tris-HCl pH 7.0	1 ml
Glucose-1-phosphate	6 mg
Glucose-1,6-diphosphate	0.04 mg
Glucose-6-phosphate dehydrogenase	0.004 ml (400 U/ml)
NADP	0.2 mg
INT	0.25 mg
PMS	trace

Esterase 3.1.1.1

Preincubate gels at 0-4°C in Tris-Maleate pH 5.3 for 15 minutes.

Tris-Maleate pH 5.3	1 ml
Na ∝-Napthyl acetate	0.5 mg
Fast Red TR Salt	0.5 mg

Hemoglobin

Preincubate gels in saturated solution of Benzidine in absolute ethanol, acidified just before use with a 1 in 5 dilution of glacial acetic acid. Leave gels for 5 minutes and then place in a 5% by volume solution of H_2O_2. Bands appear immediately and then fade away.

Amylase 3.2.1.1

Preincubate gels in starch for two hours. Wash very well and then stain in Iodine.

Starch Solution

1M K_2HPO_4	33mls
1M KH_2PO_4	66 mls
NaCl	0.6 g
Starch	10 g

H_2O to a total of 1 liter

Iodine Solution

KI	16.6 g
I_2	25.4 g

H_2O to total of 2 liters.

To use, dilute 1 in 2.

Glutarate oxaloacetate transaminase 2.6.1.1

Tris-HCl pH 9.0	1 ml
Pyridoxal-5-phosphate	0.4 mg
D-l-Aspartic acid	4 mg
α-Keto-glutaric acid	2 mg
Fast Blue BB salt	3 mg

Leucine aminopeptidase 3.4.1.1

Preincubate gels at 0-4°C for 15 minutes in Tris-Maleate pH 5.3

Tris-Maleate pH 5.3	1 ml
Na-ℓ-Leucyl-β-Napthyl amide	0.5 mg
Fast Black K salt	1 mg

Alkaline phosphatase 3.1.3.1

Tris-HCl pH 9.0	1 ml
NaCl	10 mg
Polyvinyl pyrolidine	5 mg
Na-α-Napthyl phosphate	0.5 mg
Fast Blue BB salt	0.5 mg

Tetrazolium oxidase

This enzyme appears as a white band on gels stained for Lactate or Malate dehydrogenase and exposed to light.

Table 2.4 *Electrophoresis Procedures*

Enzyme	Gel Buffer and Acrylamide Concentration	Ammonium Persulphate (mg/ml)
Lactate dehydrogenase	Tris-HCl 7.5%	0.5
Malate dehydrogenase	Tris-HCl 7.5%	0.5
Xanthine dehydrogenase	Tris-HCl 7.5%	0.5
Fumarase	Tris-HCl 7.5%	0.5
Hemoglobin	Tris-HCl 7.5%	0.5
Esterase-1	Tris-HCl 7.5%	1.25
Phosphoglucoisomerase	Tris-HCl 7.5%	0.5
Phosphoglucomutase	Tris-HCl 7.5%	0.5
Glutarate oxaloacetate transaminase	Tris-HCl 7.5%	0.5
Leucine aminopeptidase	Tris-Borate 7.5%	1.25
Alkaline phosphatase	Tris-Borate 7.5%	1.25
Amylase	Tris-Borate 7.5%	0.5
Esterase-2	Tris-Borate 10%	1.25

Table 2.4 Continued.

Enzyme	Gel Buffer and Acrylamide Concentration	Ammonium Persulphate (mg/ml)	
Tetrazolium Oxidase	Tris-HCl 7.5%	0.5	
Glucose-6-phosphate dehydrogenase	Tris-HCl 7.5%	0.5	Animals must be crushed in 10 mg NADP/ml H_2O. Add 1 ml NADP solution to gels also.

CHAPTER III

GENETIC VARIATION IN NATURAL POPULATIONS OF *DAPHNIA PULEX*

CHAPTER III

INTRODUCTION

Most species of Daphnia reproduce by cyclic parthenogenesis where asexual reproduction alternates with periods of sexual reproduction. Resting eggs, or ephippia, are produced during periods of sexual reproduction and they are responsible for re-establishing populations in ponds that cannot support a Daphnia population year round. In addition they are the only means of dispersal between habitats. There is at least one sexual generation per year in most habitats, but asexual reproduction may continue uninterrupted for many generations in permanent habitats such as large ponds and lakes. Although ephippia may be produced in these habitats, they may not hatch for long periods of time.

Because of the alternation of sexual and asexual generations, the pattern of genetic variation in cyclic parthenogens should be quite different from either purely sexual or purely asexual organisms. In these organisms sex allows the production of novel genotypes, while clonal reproduction allows genotypic replication. Extended clonal reproduction would tend to maintain linkage disequilibrium. At the same time, selection against individual genotypes can be prolonged for longer periods of time Young (1979b).

To investigate the genetic effects of this breeding system Hebert (1974a,b, 1975) studied allozyme variation in 40 English populations of D. magna. He found striking genetic differences between permanent and intermittent populations. (Intermittent populations are present for only part of the year due to periods of dessication or freezing). In intermittent populations genotypic frequencies at polymorphic loci approached Hardy-Weinberg equilibrium. Gene frequencies remained stable from year to year and samples taken weekly showed that there was very little fluctuation during individual years. In this situation, D. magna behaved much like a sexually reproducing organism. The pattern in permanent populations, however, was very different. Genotypic frequencies were found to deviate significantly from Hardy-Weinberg equilibrium, usually as a result of an excess number of heterozygotes. Furthermore, rapid changes in genotypic frequencies occurred throughout the year, perhaps as a result of natural selection.

Berger and Sutherland (1978) looked at allozyme variation in another Cladoceran, D. pulex, also thought to be a cyclic parthenogen. They studied two populations, one in a large permanent lake and another in a small temporary lake in New York. Variability was found at 6 of 17 loci analysed in the permanent population. Genotype frequencies were monitored at three loci, Alk-B, Est-A and Est-B for 18 months. Significant genotype frequency changes were noted at two of the loci, Alk-B and Est-A. When genotypic frequencies at

the variable loci in this population were compared to Hardy-Weinberg expectations, it was found that Alk-B and Est-B were in equilibrium for most of the sampling period. At the former locus, three cases of small but significant heterozygote excess and one of heterozygote deficiency were found in the eight samples. Twelve samples were analysed at Est-B and only one small insignificant heterozygote deficiency was noted. In contrast, the Est-A locus was not in equilibrium in most of the 12 samples and in six cases there were large heterozygote deficiencies.

The temporary lake was sampled only once during the late spring and variation was found at 4 of 15 loci analysed (two loci seen in the permanent population did not show allozyme activity). There were large heterozygote excesses at all four of these loci, in fact, in three cases, the population was fixed for the heterozygote.

Berger and Sutherland's observations differ from Hebert's observations in several ways. First, the tendency towards homozygote excess in the permanent population of D. pulex contrasted with the frequent heterozygote excesses seen in permanent populations of D. magna. In addition, the strong excess of heterozygotes (to the point of fixation in some cases) in a temporary population, not long after the re-establishment of the population from ephippial eggs, contrasts with the Hardy-Weinberg equilibrium seen in most intermittent D. magna populations. If Berger and Sutherland's intermittent D. pulex population was indeed

founded from sexually produced ephippial eggs, the complete absence of homozygotes at four loci suggest the presence of numerous recessive lethals.

Although the two species appear to have similar life histories, these studies have shown that the pattern of genetic variation in them is quite different. So far, only two populations of D. pulex have been examined so it is difficult to say if they were the exception or the rule. In order to investigate this problem further, a study of allozyme variation in several natural populations of D. pulex from various habitats was undertaken. All the ponds examined were intermittent so that populations were refounded each year from ephippial eggs.

MATERIALS AND METHODS

Analysis of D. pulex Populations

D. pulex was collected from eleven ponds in southwestern Ontario using the method described in Chapter II. Collections were made in the early spring not long after the re-establishment of the populations from ephippial eggs. These ponds were filled with water only during the spring and early summer. In some years the ponds refill in the fall but that was not the case during the year in which this study was done. The ponds were grouped into two categories; 1) urban and farmland ponds and 2) natural woodland ponds. The first group included nine ponds and the second included Rondeau-I and Rondeau-II.

Five ponds were found in farmland areas. Cedar Springs (1), Charing Cross-I (2) and Charing Cross-II (3) were all located in open cattle pastures but Cedar Springs was surrounded by a stand of willow trees. Bloomfield (9) was located in a farmyard and Kingsville (5) was located in Jack Miner's bird sanctuary. Each of the five farmland ponds had a maximum depth of one meter and each, except perhaps Kingsville, was manmade.

Four populations were found in urban habitats. Windsor-I (6) and Windsor-II (7) were located near a wooded area behind some houses next to Ojibway Park. Windsor-I was a small weedy pond near a drainage ditch and although it contained many leaves and twigs it was exposed to full

sunlight. Windsor-II was nearby but was completely shaded by trees. As a result, it too was filled with abundant leaf litter and organic debris. It had a maximum depth of 0.5 meters. The third urban pond, Windsor-III (8) was located in a grassy area near a sanitary landfill. It had a maximum depth of one meter. The fourth pond, Cottam (4), was located in a stand of trees near the side of the road. Like Windsor-II it was well shaded and filled with leaf litter. It had a maximum depth of one meter.

The Rondeau ponds (10 and 11) were located in wooded areas of Rondeau Provincial Park. The forest in this park is of the type found in the Carolinian zone. The ponds were less than 0.5 meters in depth and were filled with twigs and leaf litter. The zooplankton communities differed from those in farmland ponds. They included species from the genus Scapholeberis and Ceriodaphnia as well as Daphnia laevis. This land has been essentially undisturbed by man. Figure 3.1 shows the location of the eleven ponds.

Ten enzyme systems; Amylase (Amy)(2 loci), Fumarase (Fum), Glutarate oxaloacetate transaminase (Got), Glucose-6-phosphate dehydrogenase (G6pdh), Lactate dehydrogenase (Ldh), Malate dehydrogenase (Mdh), Phosphoglucose isomerase (Pgi), Phosphoglucose mutase (Pgm), Tetrazolium oxidase (To) and Xanthine dehydrogenase (Xdh) were analysed electrophoretically using the methods described in Chapter II. Genotypic frequencies for each locus were determined by electrophoresing at least 48 adult females from each pond.

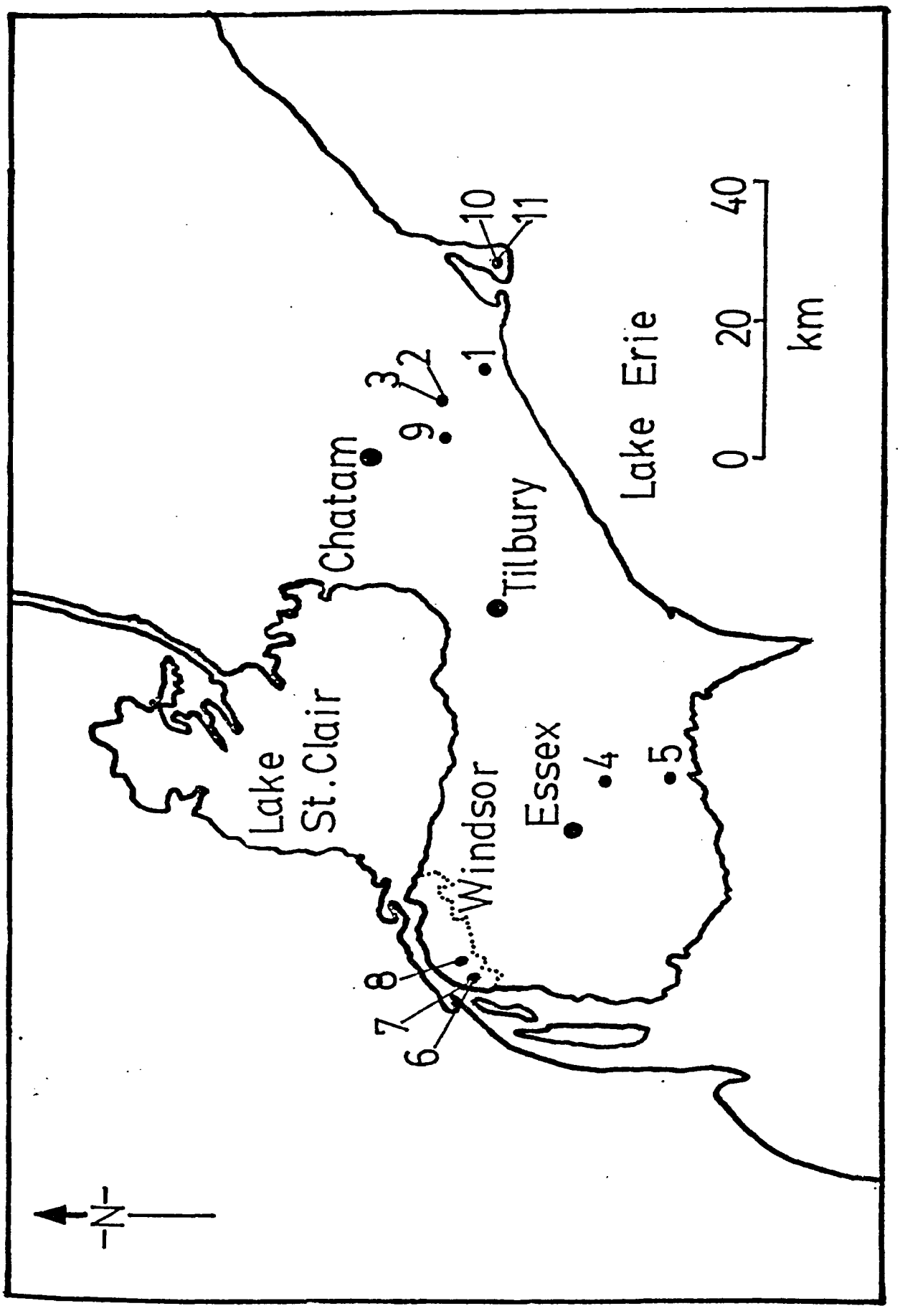


Figure 3.1 Location of D.pulex Populations

Loci were numbered in order of increasing mobility. Alleles were designated slow (S), medium (M) or fast (F) depending on their relative rate of migration from the origin. If variation was detected at a particular locus the sample size was increased to 144 or more. In addition, at least 24 females from each pond were isolated in 100 ml plastic beakers and maintained as described in Chapter II. Mortality due to transfer from pond water to artificial medium was negligible.

The laboratory clones provided data on associations between genotypes at different loci and this information was used to determine the clonal complement of each pond. Many ephippia were produced in the cultures even though males were never observed. These ephippia were hatched by air drying them in plastic beakers for several days and then flooding them with fresh artificial pond water. Hatching commenced two to three days later and continued for several weeks. Hatchlings were isolated and the offspring electrophoresed to determine if they had the same genotype as the clone that produced them.

Statistical Analysis

The data on clone genotypes were used to calculate the average heterozygosity per locus per individual. In addition, Nei's measures of genetic divergence, I and D, were calculated for each pair of clones. Because obligate parthenogenesis eliminates the possibility of interbreeding among clones, each one was considered to be a separate pop-

ulation. Nei's statistics are based on gene frequencies which, in this case, were calculated from genotypes of individual clones.

I measures how genetically similar two populations or species are to one another while D measures how genetically different they are. Consider two populations X and Y. Let x_i and y_i be the frequency of the i^{th} allele in each population. The probability that two randomly chosen genes are identical in population X is $j_x = \sum x_i^2$ and similarly for Y we have $j_y = \sum y_i^2$. The probability of identity between genes if one chosen from X and one from Y is $j_{xy} = \sum x_i y_i$. J_X, J_Y and J_{XY} are the arithmetic means of j_x, j_y and j_{xy} over all loci, including the monomorphic ones. I, the normalized identity of genes between X and Y, is given by $I = J_{XY} / \sqrt{J_X J_Y}$. I varies from zero to one. To measure the standard genetic distance we use $D = -\log_e I$. D measures the mean number of codon differences per locus and varies from zero to infinity. If we assume that the rate of gene substitution per locus is the same for all loci then D can be interpreted as the accumulated number of gene substitutions per locus (Nei, 1975). Only the codon differences that are detectable by the technique being used, in this case, electrophoresis, are measured. Not all codon changes are detectable by this technique and thus D will be underestimated.

If few loci are studied the estimate of D obtained may at best be a good approximation of the actual genetic

distance. The fewer the number of loci that are examined the more D may deviate from its real value. Nei points out that in cases where few loci are being studied D is most useful as a relative measure of genetic distance among several populations.

The unweighted pair group method of cluster analysis was performed on the matrix of genetic distances among clones in order to construct a dendogram illustrating the genetic relationship of the clones. The clustering procedure was as follows. The pair of clones with the smallest genetic distance forms the first group. A new matrix of genetic distances is then constructed in which this new cluster is considered to be a single clone. This procedure is repeated with clones either being added to existing clusters or starting new ones. The process is finished when all the clones are grouped into a single cluster. A computer program supplied by M. Nei (pers. comm.) was used to perform the analysis. A copy of this program, modified for the University of Windsor facility, is shown in Appendix I.

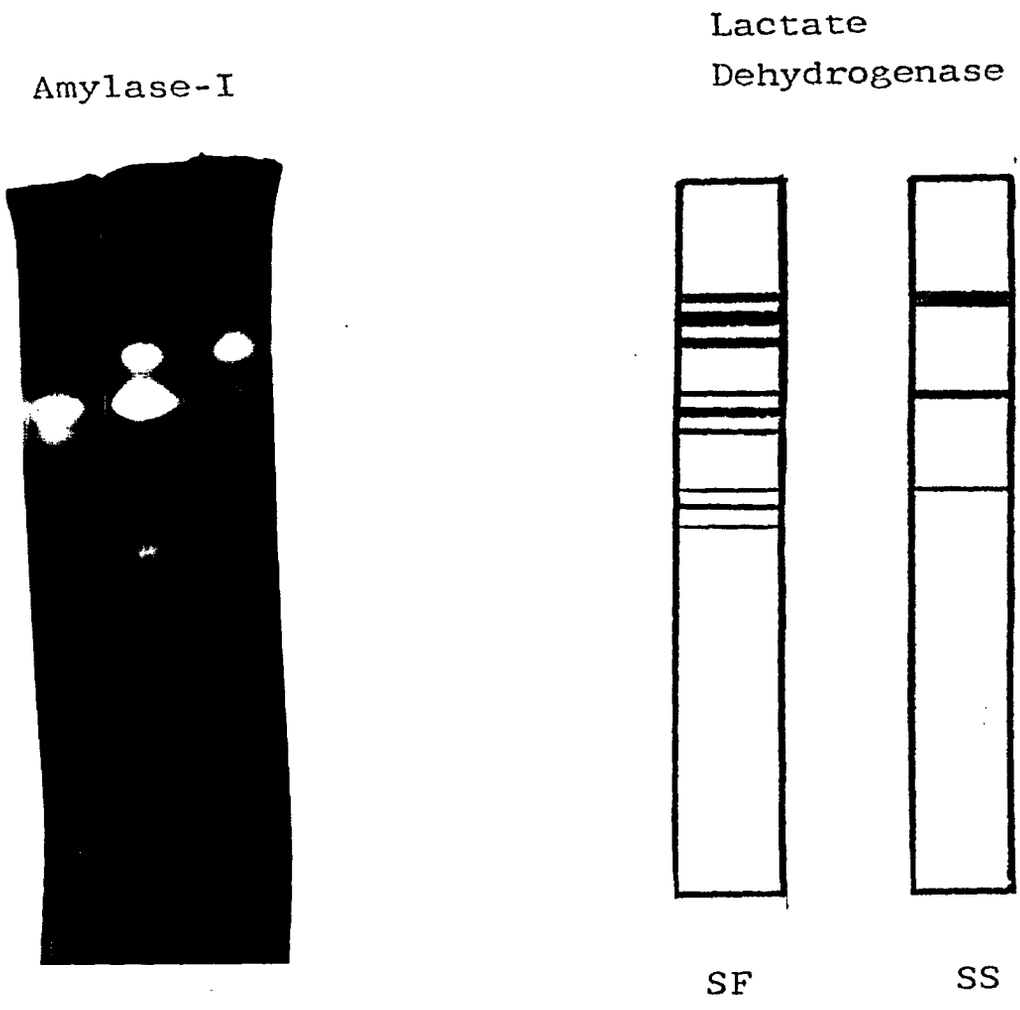
RESULTS

Fum, Xdh, G6pdh, To, Mdh, and Got were monomorphic for the same allele in all eleven populations. All of these enzymes except MDH were represented by a single band of activity on the gels. MDH showed two bands of activity. Previous work by Hebert (1972) on D. magna has shown that this pattern is characteristic of homozygotes at the locus (see Figure 4.4).

Two phenotypes were seen at the Ldh locus, a three-banded and a nine-banded pattern (Figure 3.2). Hebert (1972) had shown that individuals homozygous for Ldh show three bands. Presumably the three bands are the result of conformational changes or the binding of some charged molecule such as NAD. A similar case is seen in Alcohol dehydrogenase in Drosophila (Jacobsen, 1968). I assumed that the nine-banded pattern represents the heterozygote. The bands were in three groups of three suggesting LDH in Daphnia is a dimer. The homozygote bands coresponded to the slowest band in each triplet so it was designated the SS homozygote. The FF homozygote was not detected in any of the populations.

There were also two alleles, S and F, at the Pgi locus and all three phenotypes were detected. Homozygotes were single banded and heterozygotes were triple banded indicating a dimeric structure for PGI.

Figure 3.2 Amylase and Ldh Allozyme Patterns in D. pulex



Three alleles were found at both the Pgm and Amy-1 loci but only five of the six possible genotypes were seen. In each case the SF heterozygote was missing. Homozygotes had one band while heterozygotes had two indicating that these two enzymes were monomers. This interpretation has been confirmed for PGM from results of breeding studies in D. magna (Chapter V). Unfortunately no variation was seen at the Amy-1 locus in this species.

Amy-2 phenotypes were similar to those of Amy-1 however four alleles were detected. One of these was a null allele. Individuals homozygous for this allele had no AMY-2 activity. Only four phenotypes were seen at this locus; SM, MM, MF and Nul. Of course, in the absence of breeding studies, it was not possible to determine if individuals with the MM phenotype were actually heterozygous for the Nul allele. The AMY banding patterns are shown in Figure 3.2.

Table 3.1 shows the genotype frequencies at Ldh, Pgi, Pgm and Amy-1 for each of the populations. Because AMY-2 was difficult to score in individual animals it was ommitted here. There was much variation in genotype frequencies among populations. Deviations from Hardy-Weinberg expectations were common but the direction of these deviations was not consistent (X^2 values can be seen in Table 3.2). For example, in the Windsor I and Windsor II populations there were large excesses of heterozygotes at the Ldh locus, but heterozygotes were completely lacking at the Pgm locus.

Table 3.1 Genotypic Frequencies at Four Polymorphic Loci in Populations of *D. pulex* from Southwestern Ontario.

	LDH			AMY-1					
Population	n	SS	SF	n	SS	SM	MM	MF	FF
1. Cedar Springs	192	.005	.995	50		.02	.04	.94	
2. Charing Cross-I	72		1.00	72				1.00	
3. Charing Cross-II	48		1.00	44				1.00	
4. Cottam	309	.89	.11	117				.12	.88
5. Kingsville	120		1.00	141	.028	.035	.936		
6. Windsor-I	222	.356	.644	71		.028		.761	.211
7. Windsor-II	191		1.00	120		.658		.347	
8. Windsor-III	186	.027	.973	167			.042	.946	.012
9. Bloomfield	96		1.00	72				1.00	
10.Rondeau-I	96	1.00		72				.014	.986
11.Rondeau-II	72	1.00		48				.104	.896

Table 3.1 Continued

	PGI				PGM					
Population	n	SS	SF	FF	n	SS	SM	MM	MF	FF
1. Cedar Springs	278	.126	.126	.748	189	.646		.339	.016	
2. Charing Cross-I	134		.746	.254	118	.283	.008	.729		
3. Charing Cross-II	202		.03	.97	174	.983		.017		
4. Cottam	216		.023	.977	201	.06		.264	.677	
5.Kingsville	168			1.00	168	.006		.030	.964	
6. Windsor-I	95			1.00	287	.631		.369		
7. Windsor-II	120			1.00	217	.35		.65		
8. Windsor-III	96			1.00	216	.926		.014	.060	
9. Bloomfield	96			1.00	96			1.00		
10.Rondeau-I	96		.26	.74	72	.028		.042	.333	.597
11.Rondeau-II	48		.75	.25	47	.021	.021	.191	.745	.021

Table 3.2 Chi-square Analysis of Genotype Frequencies in the D. pulex Populations.

Population	Locus	X^2	D. F.
1. Cedar Springs	Ldh	188.03**	1
	Pgi	96.66**	1
	Pgm	183.70**	2
	Amy-1	41.03**	1
2. Charing Cross-I	Ldh	72.00**	1
	Pgi	47.43**	1
	Pgm	113.08**	1
	Amy-1	72.00	1
3. Charing Cross-II	Ldh	48.00**	1
	Amy-1	44.00**	1
4. Cottam	Pgm	42.01**	1
5. Kingsville	Ldh	120.00**	1
	Pgm	149.29**	2
6. Windsor-I	Ldh	50.16**	1
	Pgm	287.10**	1
	Amy-I	26.01**	1
7. Windsor-II	Ldh	191.00**	1
	Pgm	216.95**	1
	Amy-1	120.00**	4

Table 3.2 Continued

	Population	Locus	X^2	D.F.
8.	Windsor-III	Ldh	167.06**	1
		Pgm	8.28**	1
		Amy-I	133.54**	1
9.	Bloomfield	Ldh	96.00**	1
		Amy-1	72.00**	1
10.	Rondeau-I	Pgm	0.08	1
11.	Rondeau-II	Pgm	17.31**	2

* significant at the 0.05 level
** significant at the 0.01 level

In fact, in seven of the nine urban and farmland ponds, the Ldh-SF heterozygote was common or fixed while the Pgm-SM heterozygote was absent even though both SS and MM homozygotes were present in high frequency.

Analysis of the laboratory clones revealed definite associations between genotypes at different loci. A good example is the association between Pgm and Amy-1 genotypes in the Windsor II population. Clones that were MM homozygotes at the Pgm locus were invariably SM heterozygotes at the Amy-1 locus. Similarly, Pgm-SS homozygotes were always Amy-1-MF heterozygotes. No other combinations of these two loci were found in the population. Similar associations were also seen in the other populations. Genotypes and frequencies of the clones in each pond can be seen in Table 3.3. Note that most of the ponds contained only two or three common clones. Rondeau I and Cedar Springs contained the highest number of clones with seven each while Bloomfield had only one. Note that it was heterozygous at four of the five variable loci. Altogether 22 clones were found among eleven populations. Clone 1 was most common being found in seven out of nine urban and farmland ponds.

Table 3.3 also shows the average heterozygosity per locus per individual for each clone based on the eleven loci studied. Values ranged from zero to 0.364. The average heterozygosity was 0.153. Five clones were monomorphic at all eleven loci, three were heterozygous at one

Table 3.3 Clonal Complements of the *D. pulex* Populations

Population	Clone	Frequency	Genotype					Heterozygosity per Locus per Individual
			Ldh	Pgm	Pgi	Amy-1	Amy-2	
1. Cedar Springs	1	37	SF	SS	FF	MF	MM	0.182
	2	3	SF	MM	FF	MF	MM	0.182
	3	1	SF	MM	SS	MF	MM	0.182
	4	1	SF	MM	SS	MF	SM	0.273
	5	2	SF	MF	FF	MM	SM	0.273
	6	2	SF	MM	SF	MF	SM	0.364
	7	1	SF	MF	FF	SM	MM	0.273
2. Charing Cross-I	6	33	SF	MM	SF	MF	SM	0.364
	1	15	SF	SS	FF	MF	MM	0.182
3. Charing Cross-II	1	23	SF	SS	FF	MF	MM	0.182
	6	1	SF	MM	SF	MF	SM	0.364
4. Cottam	8	41	SS	MF	FF	FF	MM	0.091
	9	34	SS	MM	FF	FF	MM	0.00
	1	5	SF	SS	FF	MF	MM	0.182

Table 3.3 Continued

Population	Clone	Frequency	Genotype					Heterozygosity per Locus per Individual
			Ldh	Pgm	Pgi	Amy-1	Amy-2	
4. Cottam	6	5	SF	MM	SF	MF	SM	0.364
	10	11	SS	MF	FF	FF	MF	0.182
5. Kingsville	11	94	SF	MF	FF	MM	MF	0.273
	12	2	SF	MM	FF	SM	MM	0.182
6. Windsor-I	1	39	SF	SS	FF	MF	MM	0.182
	13	6	SS	MM	FF	FF	Nul	0.00
	12	2	SF	MM	FF	SM	MM	0.182
7. Windsor-II	12	58	SF	MM	FF	SM	MM	0.182
	1	38	SF	SS	FF	MF	MM	0.182
8. Windsor-III	1	68	SF	SS	FF	MF	MM	0.182
	11	3	SF	MF	FF	MM	MF	0.273
	14	1	SF	SS	FF	MM	MF	0.182
9. Bloomfield	6	48	SF	MM	SF	MF	SM	0.364

Table 3.3 *Continued*

Population	Clone	Frequency	Genotype					Heterozygosity per Locus per Individual
			Ldh	Pgm	Pgi	Amy-1	Amy-2	
10. Rondeau-1	15	27	SS	FF	FF	FF	Nul	0.091
	8	14	SS	MF	FF	FF	MM	0.091
	16	2	SS	MM	SF	FF	MM	0.091
	17	2	SS	SS	FF	FF	MM	0.00
	18	1	SS	FF	SF	FF	MM	0.091
	19	1	SS	MF	SF	FF	MM	0.182
	20	1	SS	MF	FF	MF	MM	0.182
11. Rondeau-II	19	13	SS	MF	SF	FF	MM	0.182
	8	7	SS	MF	FF	FF	MM	0.091
	17	1	SS	SS	FF	FF	MM	0.00
	21	1	SS	SM	FF	MF	MM	0.182
	16	1	SS	MM	SF	FF	MM	0.091
	22	1	SS	FF	FF	FF	MM	0.00

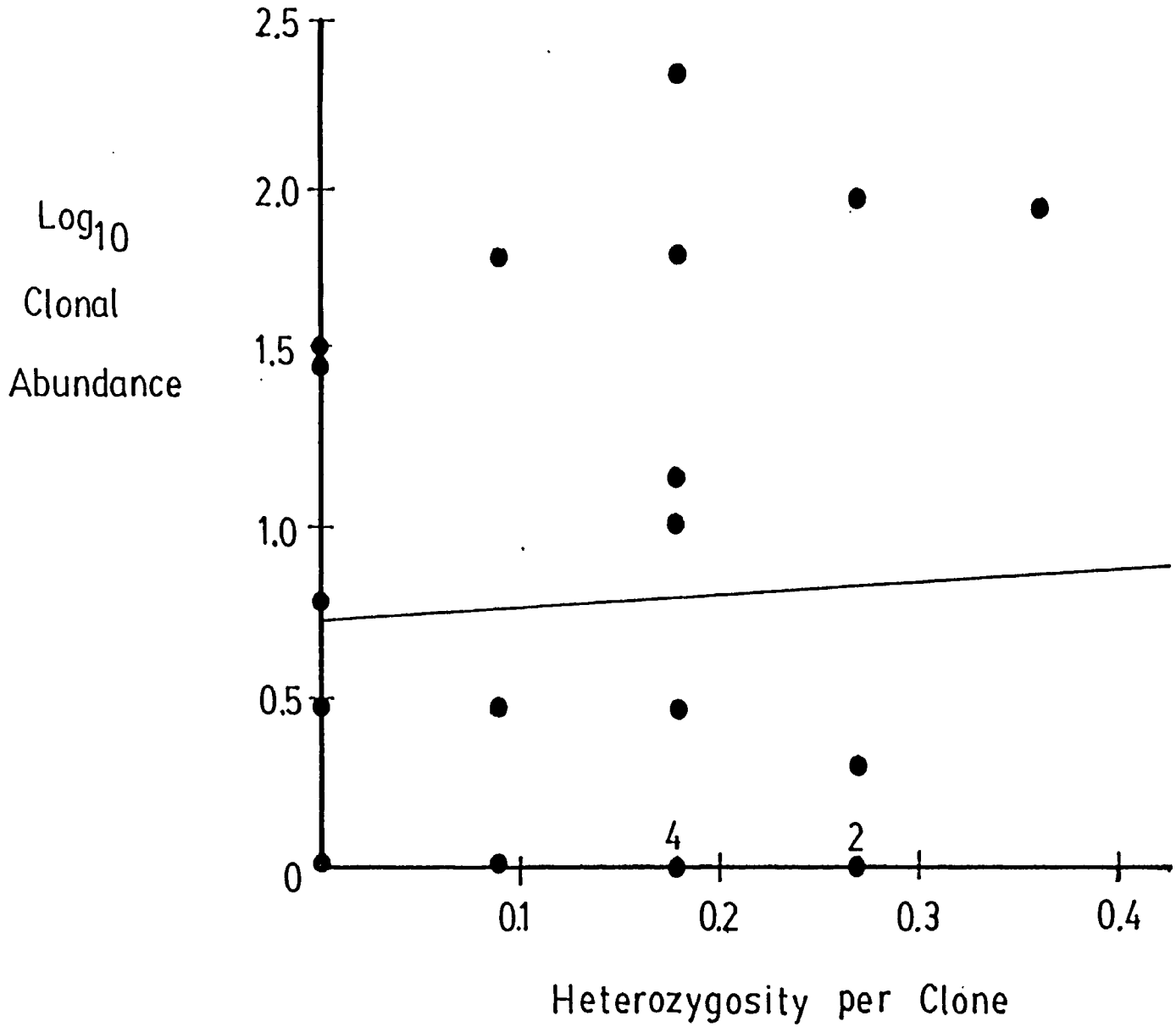
locus, nine at two loci, four at three loci and one, clone 6, was heterozygous at four loci.

Hebert's (1972) work with D. magna suggested that heterozygotes had higher fitness than homozygotes. As a result it was decided to see if there was any correlation between the heterozygosity of a clone and its relative abundance. The heterozygosity of each clone was plotted against the $\log_{10}$ of its absolute frequency (Figure 3.3). The frequencies were determined by using the data in Table 3.3. A linear regression analysis was performed on the data and a correlation coefficient of 0.051 was obtained. It was not significant at the 5% level indicating that there was no correlation between heterozygosity and relative abundance. In fact, the most common clone, clone 1, was only heterozygous at two of the eleven loci.

Two of the ponds were sampled on several occasions and temporal changes in clonal frequencies were noted. Charing Cross I was first sampled in mid May of 1978 when clone 1 made up 93% of the populations. By June 1978 the frequency of this clone had declined to 26%. This pattern was reversed in 1979 with clone 1 making up only 8% of the population in mid April and increasing to 32% by the end of the month. In mid June, the pond was sampled again and clone 1 made up 69% of the population.

When the Windsor I pond was sampled in mid April of 1979, clone 13 made up 31% of the populations and increased

Figure 3.3 The Relationship Between Heterozygosity and Abundance in the Clones of D.pulex



to 58% by the end of the month. In early May 1979, however, the clone had declined to about 28% and was absent from the population at the end of the month (Table 3.4).

Because of strong linkage disequilibrium and extreme heterozygote deficiencies it was suspected that these populations were reproducing by obligate parthenogenesis. Further study confirmed this hypothesis. For example, males had never been observed in the pond samples or in laboratory cultures even during periods of ephippia production. When 90 ephippia produced by isolated females were checked, only ten did not contain eggs. Eleven had one egg, 67 had two eggs and two had three eggs. Daphnia species reproducing by cyclic parthenogenesis are known to cast empty ephippia in the absence of males (Agar, 1920).

Forty-one offspring, hatched from ephippia produced by isolated females, were electophoresed for LDH and PGI to see if they possessed the maternal genotype. Seventeen hatchlings from Ldh-SF mothers were electrophoresed and found to be Ldh-SF as well. Another 24 hatchlings were checked at both Ldh and Pgi. Twenty-three had the same genotypes as their mothers. The 24th had the same Ldh genotype but differed at Pgi. The mother was an FF homozygote and the offspring was an SF heterozygote. The most likely explanation in this case is contamination or mislabelling of cultures as recombination could not produce an SF genotype from an FF genotype. These results suggest that the ephippial eggs are produced apomictically like the summer

Table 3.4 Temporal Changes in Clonal Frequency in Two of the *D. pulex* Populations.

a. Charing Cross-I

Clonal frequencies are based on the analysis of the *Pgi* locus in individuals taken directly from the population.

Sample Date	N	Clone 1	Clone 6	Percent Clone 1
May 25, 1978	60	56	4	93.3
June 4, 1978	47	12	35	25.5
April 16, 1979	94	19	75	20.0
June 11, 1979	96	67	29	69.8

b. Windsor-I

Clonal frequencies are based on the analysis of the *Ldh* locus in individuals taken directly from the population.

Sample Date	N	Clone 13	Clone 1+12	Percent Clone 13
April 16, 1979	103	32	71	31.1
April 26, 1979	72	42	30	58.3
May 3, 1979	96	28	68	29.2
May 9, 1979	142	38	104	26.8
May 28, 1979	144	0	144	0.0

eggs (Hebert and Ward, 1972; Zaffagnini and Sabelli, 1972) and that meiosis has been completely suppressed in the population.

The matrix of genetic similarities and genetic distances among the 22 clones is shown in Table 3.5. The mean similarity was 0.869 with values ranging from 0.977 to 0.667. Genetic distances ranged from 0.404 to 0.022 with a mean of 0.145 suggesting that an average of 14 electrophoretically detectable allele substitutions per 100 loci have occurred between any two clones.

The dendogram constructed from the genetic distance values is shown in Figure 3.4. Woodland (WL) clones (those found in the two Rondeau ponds) are marked with a '+' to distinguish them from the Urban and Farmland (UF) clones. The most divergent group was clones 13 and 15. The divergence seems to be due to their unusual Nul genotype at the Amy-2 locus. Another divergent group of clones is the 3, 4, 6 cluster. Two of these clones have the rare Pgi-SS genotype and all three possess the relatively uncommon Amy-2-SM genotype. Another small group, 1, 17 and 14, is set apart by its rare Pgm-SS genotype. The rest of the clones seem to be divided into two large groups, one consisting of five UF clones and the other consisting of seven WL clones plus clones 9 and 10. It is important to note that these two clones possess the Ldh-SS genotype which is seen in every WL clone. In fact, the major difference between these two groups seems to be their Ldh genotype. All

clones in the smaller cluster (2, 12, 5, 7, 11) are Ldh-SF while all the clones in the largest group (8, 20, 21, 10, 9, 16, 18, 22, 19) are Ldh-SS. Only one cluster, 1, 17, 14, contained both and Ldh-SS and an Ldh-SF clone.

Table 3.5 Genetic Similarity (above diagonal) and Genetic Distance (below diagonal) Between Clones of *D. pulex*

Clone	1	2	3	4	5	6	7	8	9	10	11
1	-	0.9	0.8	0.77	0.872	0.843	0.898	0.878	0.858	0.85	0.872
2	0.105	-	0.9	0.872	0.923	0.949	0.949	0.927	0.953	0.9	0.923
3	0.223	0.105	-	0.975	0.821	0.949	0.846	0.83	0.858	0.8	0.821
4	0.261	0.137	0.026	-	0.842	0.973	0.815	0.801	0.831	0.795	0.816
5	0.137	0.08	0.198	0.172	-	0.919	0.947	0.851	0.831	0.846	0.974
6	0.17	0.053	0.053	0.023	0.084	-	0.892	0.874	0.904	0.87	0.892
7	0.108	0.052	0.167	0.204	0.054	0.114	-	0.901	0.88	0.872	0.947
8	0.13	0.076	0.187	0.222	0.161	0.134	0.104	-	0.977	0.976	0.851
9	0.153	0.048	0.153	0.185	0.185	0.1	0.127	0.023	-	0.953	0.831
10	0.163	0.105	0.223	0.229	0.167	0.14	0.137	0.024	0.048	-	0.872
11	0.137	0.08	0.198	0.204	0.027	0.114	0.054	0.161	0.185	0.137	-
12	0.134	0.025	0.133	0.167	0.08	0.081	0.026	0.13	0.099	0.163	0.08
13	0.271	0.153	0.271	0.245	0.245	0.158	0.245	0.123	0.095	0.099	0.245
14	0.051	0.163	0.288	0.296	0.108	0.202	0.137	0.248	0.271	0.223	0.08
15	0.271	0.27	0.404	0.379	0.245	0.283	0.245	0.123	0.201	0.099	0.245
16	0.187	0.075	0.076	0.104	0.222	0.077	0.161	0.049	0.023	0.076	0.222
17	0.478	0.153	0.271	0.31	0.245	0.218	0.185	0.072	0.095	0.099	0.245
18	0.187	0.187	0.187	0.222	0.222	0.195	0.161	0.049	0.123	0.076	0.222
19	0.163	0.105	0.105	0.137	0.198	0.11	0.137	0.024	0.048	0.051	0.198
20	0.105	0.051	0.165	0.198	0.08	0.11	0.052	0.024	0.048	0.051	0.08
21	0.051	0.051	0.165	0.198	0.108	0.11	0.08	0.05	0.048	0.078	0.108
22	0.153	0.153	0.271	0.31	0.185	0.218	0.127	0.023	0.095	0.048	0.185

Table 3.5 Continued

Clone	12	13	14	15	16	17	18	19	20	21	22
1	0.875	0.763	0.95	0.763	0.83	0.953	0.83	0.85	0.9	0.95	0.858
2	0.975	0.858	0.85	0.763	0.927	0.858	0.83	0.9	0.95	0.95	0.858
3	0.875	0.763	0.75	0.667	0.927	0.763	0.83	0.9	0.85	0.85	0.763
4	0.846	0.783	0.744	0.685	0.901	0.734	0.801	0.872	0.821	0.821	0.734
5	0.923	0.783	0.898	0.783	0.801	0.783	0.801	0.821	0.923	0.898	0.831
6	0.922	0.854	0.817	0.754	0.926	0.804	0.823	0.896	0.896	0.896	0.804
7	0.975	0.783	0.872	0.783	0.851	0.831	0.851	0.872	0.949	0.923	0.88
8	0.878	0.884	0.781	0.884	0.952	0.93	0.952	0.976	0.976	0.952	0.977
9	0.906	0.909	0.763	0.818	0.977	0.909	0.884	0.953	0.953	0.953	0.909
10	0.85	0.905	0.8	0.906	0.927	0.906	0.927	0.95	0.95	0.925	0.953
11	0.933	0.783	0.923	0.783	0.801	0.783	0.801	0.821	0.923	0.898	0.831
12	-	0.81	0.85	0.715	0.878	0.81	0.781	0.85	0.925	0.925	0.81
13	0.21	-	0.715	0.909	0.884	0.818	0.791	0.858	0.858	0.858	0.818
14	0.163	0.335	-	0.715	0.732	0.858	0.732	0.75	0.85	0.9	0.763
15	0.335	0.095	0.335	-	0.791	0.818	0.884	0.858	0.858	0.81	0.909
16	0.13	0.123	0.312	0.235	-	0.884	0.905	0.976	0.927	0.927	0.884
17	0.21	0.201	0.153	0.201	0.123	-	0.884	0.906	0.906	0.953	0.909
18	0.248	0.235	0.312	0.123	0.1	0.123	-	0.976	0.927	0.878	0.977
19	0.163	0.153	0.288	0.153	0.024	0.099	0.024	-	0.95	0.925	0.953
20	0.078	0.153	0.165	0.153	0.075	0.099	0.076	0.051	-	0.975	0.953
21	0.078	0.153	0.105	0.21	0.075	0.048	0.13	0.078	0.025	-	0.906
22	0.21	0.201	0.271	0.095	0.123	0.095	0.023	0.048	0.048	0.099	-

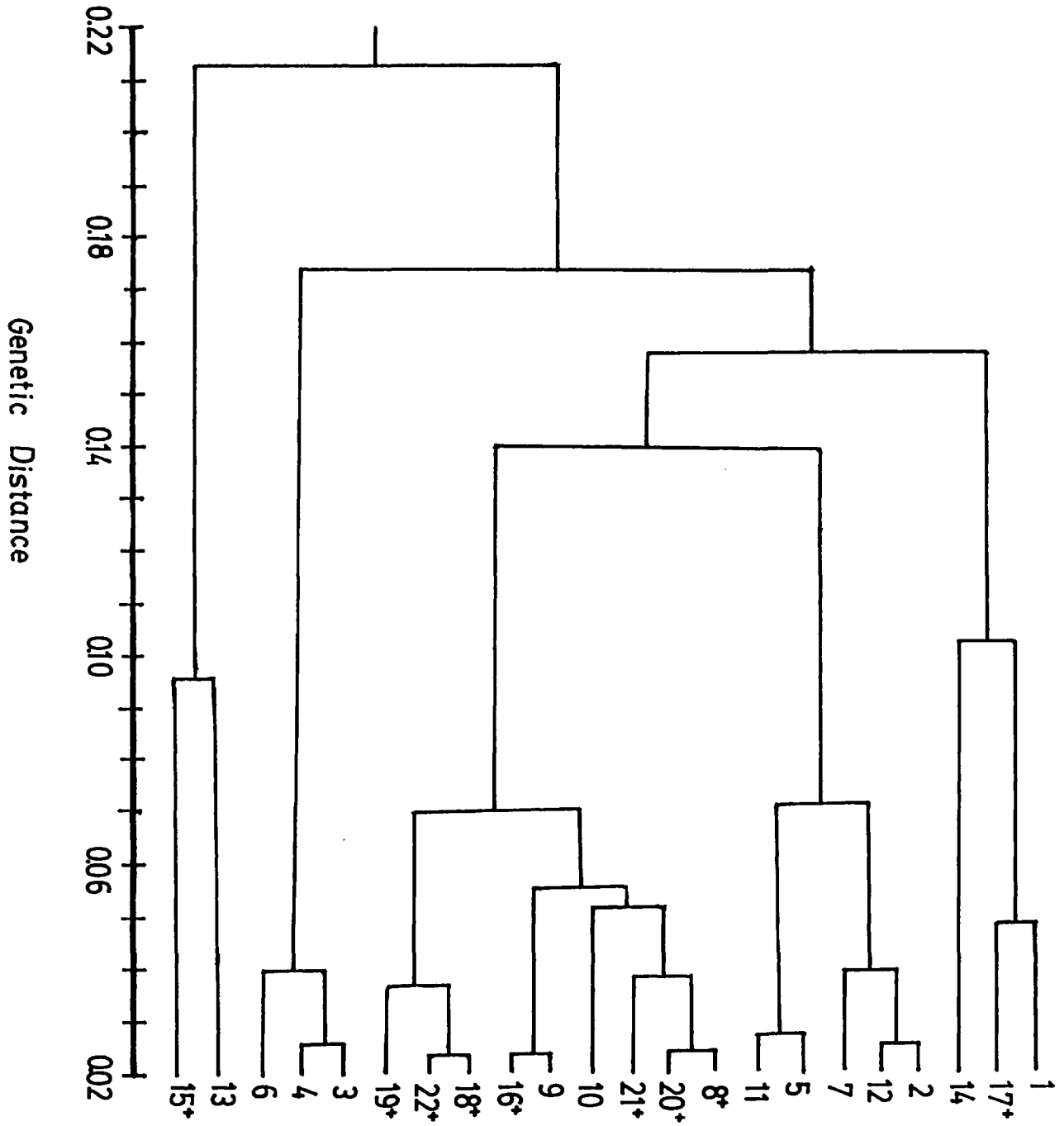


Figure 3.4 Dendogram Showing the Genetic Relationship Among Clones of D. pulex

DISCUSSION

It has been suggested that apomictic parthenogenesis will lead to an increase in heterozygosity per individual due to the accumulation of mutations (White, 1970). If this is the case one would expect obligate parthenogens such as D. pulex to be heterozygous at more loci than cyclic parthenogens such as D. magna. The results of this study support that hypothesis. The D. pulex clones were, on average, heterozygous at about 15% of their loci. Subsequent work on additional loci such as alkaline phosphatase, esterase and leucine aminopeptidase has revealed even more variation (Hebert, pers. comm.). In contrast, individual D. magna from populations in England were heterozygous at about 6.6% of their loci and individuals from the Arctic populations had virtually no heterozygosity (Chapter V). The average heterozygosity per individual in 15 populations of another cyclic parthenogen, D. carinata, from Australia was only 2.1% (Hebert and Moran, 1980).

Similar work on a variety of other parthenogens has revealed a different pattern. In general, parthenogenetic races or species seem to possess the same amount of genetic diversity as their sexual relatives. Parker et al. (1977) studied populations of the apomictic cockroach, Pycnoscelus surimanemsis, from various locations around the world and found that diploid clones of this species were

heterozygous at about 10.8% of their loci. Samples of its bisexual relative, P. indicus, showed heterozygosities of about 10.7%.

Triploid and tetraploid clones of the beetle, Otiorrhynchus scaber, have heterozygosities of 31.4% and 31.7% respectively. Bisexual, diploid populations of this species had heterozygosities of 30.9% (Suomalainen and Saura, 1973). Similarly, diploid clones of Solenobia triquetrella, a moth, were heterozygous at 25% of their loci in the case of XY clones and 20% in the case of XO clones. Tetraploid clones were heterozygous at 20.3% of their loci. These values were very similar to the value of 23% that was calculated for bisexual populations of S. triquetrella (Lokki, et al., 1975).

All of the parthenogenetic races or species described above were derived from a single ancestral sexual species rather than by the hybridization of two separate species (as in the case of the parthenogenetic lizard, Cnemidophorus tesselatus). Many people have suggested that in situations where hybridization was not involved the degree of genetic diversity in asexual populations reflects the degree of genetic variability in the ancestral species and thus accounts for the high level of variability in the parthenogenetic descendants. The similarity in heterozygosity values between parthenogenetic species and their close sexual relatives seem to support this idea.

The presence of more than one genetically distinct genotype within individual habitats was a common occurrance. All but one of the eleven D. pulex populations had two or more clones. The woodland ponds, Rondeau I and II, and Cedar Springs had the greatest number of clones with seven, six and seven respectively while the farm ponds tended to have a very low number of clones. For example, Charing Cross I and II and Kingsville each had two clones and Bloomfield had one. The paucity of clones in farmland ponds may simply be the result of founder effects.

The study of several populations of other parthenogenetic organisms has also revealed that more than one clone is often found in a single habitat. For example, in the beetle, O. scaber, 15 clones were found in three populations of triploids and as many as 9 were found in a single population. A study of six populations of tetraploids yielded 27 clones with as many as 8 in a single population. Similarly, in the moth, S. triquetrella, five populations of XY diploids had 14 clones with 1 to 5 clones per population and five populations of XO diploids had 9 clones with 1 to 5 per population. Parker and Selander (1976) found up to four clones in populations of the lizard, C. tesselatus. In addition, Jaenike, et al. (1980) found 8 clones of the earthworm, Octolasion tyrtaeum, with 2 or more clones being found in 13 of the 64 sample locations.

The magnitude of the genetic similarity values between

clones ($\bar{I}$=0.869 (0.977-0.667)) falls into the range of subspecies and semispecies of Drosophila willistoni ($\bar{I}$= 0.8 (0.77-0.83)) (Ayala, et al., 1974) and of semispecies of the genus Peromyscus ($\bar{I}$= 0.84) (Zimmerman, et al., 1979). The reproductive isolation between clones due to obligate parthenogenesis has allowed genetic differences of the same magnitude as those seen in separate species of other organisms to develop.

Jaenike, et al. (1980) calculated genetic similarities among clones of the earthworm, O. tyrtaeum, from various habitats in Tennessee and North Carolina. Eight clones were identified with two being very common in a variety of habitats. Average genetic similarity between clones of this organism was 0.76 with values ranging from 0.97 to 0.33. Some of the clones in this species, then, were as dissimilar as morphologically distinct species of Drosophila.

These studies indicate that clones of parthenogenetic organisms can accumulate large genetic differences even to the point where they are as different from one another as are distinct species in other groups of organisms. Subsequent analysis of the D. pulex clones at other loci such as esterase, alkaline phosphatase and leucine aminopeptidase has shown additional differences between clones (Hebert, pers. comm). This will cause the genetic similarity values to be even lower than I have calculated on the

basis of the eleven loci studied to date. This additional analysis has not revealed the existance of any new clones suggesting that perhaps we are approaching the upper limit to the number of clones in this area.

Both mono and polyphyletic origins have been proposed for the clonal diversity noted in parthenogenetic organisms. Suomalainen and Saura (1973) have suggested a monophyletic origin for the clones seen in asexual populations of the weevil, O. scaber. They show that all the variation seen among clones can be explained as deviations from one ancestral type either through recombination or mutation. Recall that they found 15 triploid and 27 tetraploid clones in the nine populations they sampled. This shows that while evolution may be slower in asexual organisms, they still retain much potential for genetic change, even after the ability to reproduce sexually is lost.

Several people have proposed a polyphyletic origin for parthenogenesis in a variety of other asexual organisms including the cockroach, P. surinamensis, the moth, S. triquetrella, and a variety of earthworm species in several genera (Jaenike and Selander, 1979). The array of clonal genotypes in populations of these species cannot be simply explained as evolving from one single ancestral type. In such a situation asexual reproduction probably evolved independently in several different lines.

The strong linkage disequilibrium, frequent gene sub-

stitution and relatively low genetic similarities among clones of D. pulex within habitats strongly suggests that they did not evolve within these particular habitats but were carried in from outside. However, a monophyletic origin of the clones cannot be ruled out. It is possible that all these clones originated from a single population and were then dispersed to other habitats.

Several people have formulated theories to explain how asexual reproduction could evolve in one clone and then spread throughout the population and even on to other populations. Richards (1973) found that apomictic species of the dandelion, Taraxacum, could still produce fertile pollen. When he used this pollen to fertilize eggs of a sexual species he obtained a fertile apomict. This suggests that pollen from an asexual species could spread obligate parthenogenesis to other species.

Jaenike and Selander (1979), working with hermaphroditic earthworms, and Hebert (1978, in prep), working with Daphnia, independantly developed a similar hypothesis for the spread of parthenogenesis in animals. First of all, a dominant mutation which suppresses meiosis in the production of eggs but not in the production of sperm arises. In hermaphrodites the individual possessing this mutation produces its eggs asexually but still produces normal sperm. During mating with another individual it releases its own sperm (50% of which carry the mutation for par-

thenogenesis) to fertilize the other's eggs but the sperm it receives are not used. In the next generation 50% of the offspring of the normal individual will be parthenogenetic. In this way the gene for parthenogenesis will spread throughout the population until no hermaphroditic individuals remain.

Hebert's hypothesis for Daphnia is very similar. In this case the dominant mutation arises in a single female in a population of a cyclic parthenogen. As a result this female produces her ephippial eggs asexually but is still able to produce normal, fertile males. Her male offspring (50% of their sperm carry the mutation) will mate with normal females which produce haploid sexual ephippial eggs. As a result, 50% of the females that hatch from these ephippia will be obligate parthenogens. If ephippia of obligate females are transferred to other ponds, the male offspring of these hatchlings will spread the mutation through this population as well. Hebert determined through a computer simulation that if a single copy of this gene was introduced into a cyclic population through ephippial transfer, the gene would go to fixation very quickly. If the population size was 10^6, it took approximately 50 generations to reach fixation.

Both models will work equally well if a recessive mutation is proposed however the complete conversion to obligate parthenogenesis will take many many more generations.

Support for the hypothesis in Daphnia comes from the fact that obligate parthenogenetic species such as D. middendorffiana and D. cephalata retain the ability to produce males. In addition, many parthenogenetic earthworms retain male-related structures (Jaenike and Selander, 1979). Furthermore, Carson (1967) has shown that the ability to reproduce asexually in Drosophila mercatorum, a normally sexual species, can be transmitted through the male parent.

This theory explaining the spread of obligate parthenogenesis helps to explain the relationship between the D. pulex clones shown in the dendogram in Figure 3.4 if we imagine that genotypes are "frozen" as obligate parthenogenesis spreads through a population. Generally the woodland clones were more closely related to one another than to the urban and farmland clones and vice versa. One would expect populations in one area to be more similar to each other than to populations in other areas. This was certainly the case in D. magna populations from England and the Arctic (Chapter V and Hebert, 1972). It follows then, that the clones from neighbouring ponds should be more similar to one another than to the clones from other areas. Note that in most cases the tightest cluster contained clones from the same or neighbouring ponds. All the clones in the 3, 4, 6 cluster were found in the Cedar Springs pond. Similarly the 8, 20, 21 cluster and the 18, 22, 19 cluster contained clones found in the Rondeau

ponds. The associations are far from perfect but a general trend does seem to exist.

In looking at the distribution of D. pulex clones, an interesting correlation between the Ldh genotype and habitat type became apparent. Clones with the SF genotype were found in urban and farmland ponds while SS homozygotes were found in natural ponds. The SF genotype is associated with a variety of very diverse genotypes suggesting that the F allele is quite old; it probably existed before the populations adopted obligate parthenogenesis. Furthermore, SF individuals were found in a preliminary study of populations of D. pulex from Alberta suggesting that the allele is not unique to southwestern Ontario. It is possible that natural selection is responsible for the association between habitat and Ldh genotype. The SS genotype seemed to be associated with ponds in areas surrounded by trees, with abundant leaf litter and organic debris. The SF genotype seemed to be associated with ponds in areas disturbed by man and his activities such as farming. Two urban and farmland ponds, Cottam and Windsor-I, did contain SS homozygotes however. The Cottam pond was located near the side of a road in a stand of trees and was thus filled with leaves. It was quite shallow. Windsor-I was located near a wooded area and some houses. It contained much leaf litter and was also quite weedy. The Cottam pond was most similar to the woodland ponds and SS clones (there were

three) were much more abundant than SF clones; they made up about 90% of the population. The Windsor-I population only had one SS clone which made up only about 13% of the population.

A study of ten other populations located near Kingston, Ontario (another undisturbed habitat) showed the same association. Only the SS genotype was detected in forest areas but the SF genotype was found in a population in the town of Odessa.

Several other cases of a correlation between allozyme genotype and environment have been noted in other organisms. Saul, et al. (1978) noted a direct relationship between the ratio of two esterase alleles in larvae of the mosquito, Aedes triseriatus, and the type of tree on which they were located. On beech trees the ratio of the .90 allele to the .87 allele was approximately two while on oak trees the ratio was close to one. Because many populations with different genetic histories showed the same relationship, they proposed that selection was acting on the Esterase locus itself. Similarly, Redding and Schreck (1979) found a correlation between the Isocitrate dehydrogenase (Idh) genotype of steelhead trout fry and their tolerance to high temperature and low oxygen tension. Experiments showed that the Idh-A^3A^3 genotype was more tolerant to these conditions than were AA or AA^3 individuals. They also pointed out that the A^1 and A^3 alleles were at a much

higher frequency in interior river systems whose temperatures may approach 25°C than in coastal river systems whose temperature maxima are usually close to 15°C. Furthermore, Moon and Hachachka (1972) demonstrated differential temperature dependency for the reaction kinetics of IDH isozymes in rainbow trout. Therefore Redding and Schreck proposed that selection was acting on this locus to maintain different alleles in different environments.

No work has, as yet, been done to test the hypothesis of natural selection with regard to the Ldh genotypes of D. pulex however it would be worthwhile to determine experimentally if differences in tolerance to certain environmental conditions do exist among genotypes. Competition experiments between a variety of SS and SF clones in a wide range of different conditions would provide some insight into this problem.

The fact that more than one clone was able to coexist in a single population seems to contradict the principle of competitive exclusion. The clones of D. pulex probably use identical food items; collection of food is non-selective as all or most particles of suitable size that are trapped by the filtering appendages would be ingested. Many people have shown that populations of Daphnia are food limited (Slobodkin, 1954; Hall, 1964) making food the limiting resource and therefore, the major object of competition. Competition theory states that two species compet-

ing for the same limiting resource cannot stably coexist in the same habitat. The species best able to acquire and utilize the limiting resource should eventually exclude all others. William (1975) has stressed that extended competition among clones of the same species should eliminate all but one due to the severity of resource overlap. The longer two genotypes are in competition the more likely it is that one will eventually exclude the others. This is clearly not the case in the D. pulex populations. Up to seven clones were found in one pond. Similarly Hebert(1977) found that up to three or four morphologically and genetically similar species of the Daphnia carinata complex often coexisted in lakes and ponds in Australia. Young (1979a) recognized 29 genetically distinct clones of D. magna in a permanent population in England. How then, are these clones able to coexist in the face of such strong interclonal competition?

One feature that all of these cases had in common was temporal instability of the relative frequencies of each clone or species in a particular community. Table 3.4 showed that the frequencies of the different D. pulex clones changed dramatically during a single year and also from year to year. Similarly Young (1979a) described significant gene frequency changes at several polymorphic loci in his permanent D. magna population. This suggests that the relative fitnesses of the clones were unstable, each with maximum fitness in a different set of environ-

mental conditions. As the environment changes some clones are favored and their numbers increase, while others decrease as conditions become unfavorable for them thus preventing competitive exclusion of all but one clone. In order for clonal coexistence to continue the average or long term fitness of each clone must be the same. This situation is intrinsically unstable and eventually one would expect one or more clones to be lost from a population. But, this mechanism can allow coexistence of clones indefinitely if one further condition is met; that not all the ephippia produced by each clone hatch the year after they are produced. If this is true, and the extremely large number of ephippia seen in ponds makes it likely that this is the case, a clone need never be lost from a habitat even if there are one or more years when conditions are not favorable for its growth. As long as its ephippia are present in the pond, the clone will be able to re-establish itself in years when conditions are more suitable.

Snell (1979) has shown, in laboratory competition experiments with the rotifer, Asplanchna girodi, that the clone with the highest feeding rate, assimilation efficiency and metabolic efficiency always outcompetesclones that are inferior in these characteristics when competition is for food. In this case the limiting resource, food, seemed to also be the primary determinant of fitness. But, if the limiting resource is not the primary determinant of fitness

there should be no limit to resource overlap (Hebert and Crease, 1980). Fitness differences between clones or species of Daphnia may be the result of differing suseptibility to physical factors such as pH, oxygen tension or temperature. Even though species or clones are competing for food, other factors may intervene before the exclusion of all but one clone occurs.

Several studies have indicated that temperature may be an important factor in structuring many zooplankton communities. Hairston and Kellerman (1965) found that two different strains of Paramecium aurelia preferred different temperatures; variety 2 preferred cooler temperatures than variety 3. Similarly Tappa (1965) and Hebert (1977) found that different species of Daphnia predominated in different seasons, some preferring warm temperatures and others cool temperatures. There is some evidence that temperature may also affect fitness in the clones of D. pulex. Loaring (pers. comm.) studied intrinsic rates of increase (r) and competitive ability at three temperatures (10, 20 and 30°C) in four of the D. pulex clones (1, 4, 6 and 13) described in this study. Significant differences in 'r' were noted at all three temperatures, particularly at 30°C where both the highest and lowest 'r' values of the whole experiment were seen. In addition, clones that did well in one temperature did not seem to do as well in others. For example, clone 13 had the highest 'r' value at 20°C but had the low-

est at 30°C in the experiments where Scendedesmus was used as a food source. Temperature was also seen to affect competitive ability. When clone 4 and clone 6 were competed at 10°C, clone 6 always won. At 20°C however, the two clones persisted for the length of the experiment and at 30°C clone 4 outcompeted clone 6. These results show that the different clones were not selectively neutral and that temperature plays a significant role in affecting their relative fitnesses.

Other workers have also suggested that coexistance can occur even in the face of extreme resource overlap. Levins (1979) stated that "a community which would not reach a stable equilibrium may nevertheless persist if there is temporal variation and non-linear (population) dynamics". These non-linearities can arise from such factors as multiple nutritional requirements or seasonally variable feeding rates. Ayala (1972) argued that if competitive fitnesses were frequency dependent, so that uncommon species have the advantage, coexistance may occur even though there is resource overlap.

Further study, both in the laboratory and in the field, of the structure of the D. pulex populations described here will provide additional insight into the mechanism of clonal coexistance. One advantage to this system is that individuals of a clone, unlike a species, are genetically identical and should respond similarly in a given set of

circumstances. To date, competition theory has dealt with species as though every individual were identical, ignoring the fact that large amounts of genetic variability do exist in most organisms. Studies of clonal interactions will allow these theories to be tested more precisely than is possible with sexually reproducing organisms.

The demonstration of obligate parthenogenesis in all the populations of D. pulex in the present study as well as in populations from Kingston, Ontario and Churchill, Manitoba (Hebert, pers. comm.) suggests that it may be much more widespread than was once thought. The unusual genetic characteristics of the D. pulex populations studied by Berger and Sutherland (1978) (see Chapter I) suggested that these populations were obligate parthenogens as well. Marked Hardy-Weinberg disturbances in populations recently established from ephippia suggest that the populations were not reproducing by simple cyclic parthenogenesis.

The results of Smith and Fraser's (1976) work on three populations of another Cladoceran, Simocephalus serrulatus, suggest that their ephippial eggs may also be produced asexually. Extreme heteozygote deficiencies were noted at most polymorphic loci in all three populations. The two common clones in one lake were homozygous for different alleles at four of five variable loci. Hebert (in prep) has suggested that perhaps S. serrulatus females are able to self-fertilize, accounting for the large heterozygote deficiency,

but retain the ability to outcross, accounting for the small numbers of heterozygotes that were observed. The genotype frequencies suggest that in these populations asexual predominates over sexual reproduction.

Most Cladocerans, with a few exceptions, are thought to be cyclic parthenogens (Pennak, 1953; Barnes, 1968) but the preceding examples have shown that further study is likely to reveal the prevalence of obligate parthenogenesis. Studies of other groups of organisms such as earthworms (Jaenike and Selander, 1979) and insects (White, 1970) have revealed that asexual reproduction is much more common in animals in general than was previously believed. Further study of the population ecology and genetics of these parthenogenetic organisms and their sexual relatives should lead to a better understanding of the forces responsible for the prevalence of sexual reproduction in the animal kingdom.

SUMMARY

Allozyme variation at 11 loci was studied in 11 populations of D. pulex from southwestern Ontario. Nine of the populations were found in urban and farmland habitats while two were located in natural woodland. Five of the loci surveyed were polymorphic. Marked deviations from Hardy-Weinberg expectations were common and there were many cases of heterozygote as well as homozygote excess, often in the same population. This suggested that these populations, thought to be cyclic parthenogens, were actually reproducing by obligate parthenogenesis. Ephippia that were produced in the absence of males were successfully hatched confirming this hypothesis. The genotypes of offspring hatched from ephippia were identical to those of their mothers indicating that the eggs were produced apomictically.

Multi-locus analysis of laboratory clones from each population revealed numerous interlocus associations. Altogether 22 distinct clones were recognized. The average number of clones per pond was 3.6 with numbers ranging from one to seven clones per pond. Nei's genetic distance was calculated for each pair of clones. The mean distance was 0.145 with values ranging from 0.404 to 0.022. A dendogram showing the genetic relationship among the clones was constructed using the genetic dis-

tance values. Generally the woodland clones were genetically more similar to one another than to urban and farmland clones and vice versa.

The coexistance of more than one clone in a habitat seems to contradict the theory of competitive exclusion. It has been suggested that competitive exclusion should be especially likely in the case of competiting clones yet clonal diversity in these Daphnia populations is high. Several mechanisms for the coexistence of clones are discussed.

CHAPTER IV

GENETIC VARIATION IN NATURAL POPULATIONS OF *DAPHNIA MAGNA*

CHAPTER IV

INTRODUCTION

It has been suggested that the morphological uniformity of pond dwelling organisms over wide geographic ranges is evidence of slow evolutionary differentiation. Mayr (1963) attributes this lack of geographic variation to the fact that dispersal in these organisms is so great that species are "virtually panmictic regardless of the geographic extent of (their) range". This suggests that the genetic composition of these species should be uniform across their ranges as well. In order to test this idea Hebert (1975) undertook a study of allozyme variation in 40 populations of Daphnia magna from three separate areas in England. Several aspects of its biology make D. magna particularly interesting. It has an extensive geographic range and yet individuals from areas as far apart as North America and Europe are virtually indistinguishable (Brooks, 1957). Furthermore, D. magna reproduces by cyclic parthenogenesis (see General Introduction). Colonization of new habitats requires the passive dispersal of ephippia by birds, animals or the wind.

Hebert (1975) predicted that if gene flow between ponds was very high, gene frequencies in any one area should be similar. His study revealed, however, that ext-

ensive genetic differentiation had occurred. Gene frequencies at polymorphic loci were very different among neighbouring ponds and gene substitutions were fairly common. Hebert suggested that gene flow between ponds was extremely limited and that ponds were likely colonized by only one or a few ephippia. As a result each individual population would contain only a fraction of the variability present in the population from which it originated due to founder effects. In addition, inbreeding would be enhanced due to the small number of clones in a pond which would further reduce the amount of variation in the population.

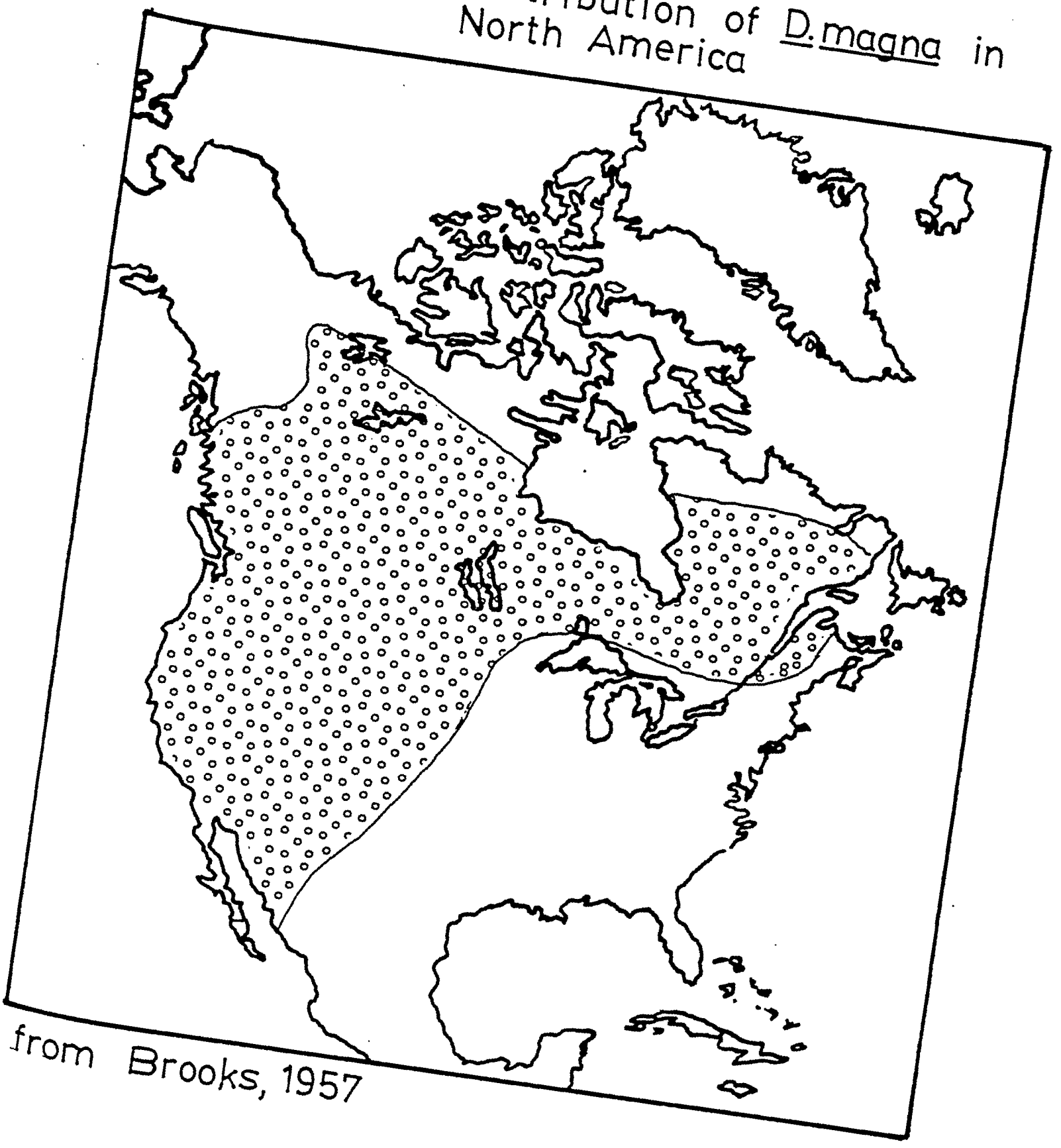
The result of this is that the gene pool in any one area is fragmented into many isolated demes with much of the variation being maintained as differences between demes. Even so, gene frequencies at polymorphic loci remained fairly stable suggesting that natural selection was acting to resist the tendency of inbreeding to eliminate variability when it did exist.

If gene flow between ponds meters apart is very low it is conceivable that gene flow between Europe and North America has been virtually non-existant for several million years. Brooks (1957) has suggested that Daphnia populations in North America may have been founded from populations that persisted in unglaciated areas of central Alaska during the last glacial advance of the Pleistocene. He suggested that these ancestral populations may have

originated from Asia and Russia. The English populations on the other hand most likely originated from western Europe. If this is the case North American and European *Daphnia* have been isolated from one another for extremely long periods of time.

D. magna is found sporadically across much of northern Canada, a habitat similar in some ways to parts of northern Europe. The present study was designed to determine how much genetic differentiation has occurred between the English populations studied by Hebert and a sample of populations from northern Canada. A comparison of these two groups is particularly interesting because both are located near the margins of the species range on their respective continents. The English populations are found on the western edge of the Old World range while the Canadian populations are near the eastern limit of the range in North America. Figure 4.1 shows a map of the distribution of *D. magna* in North America.

Figure 4.1 Distribution of D.magna in North America



MATERIALS AND METHODS

Ten ponds from the Churchill, Manitoba area were chosen for study. A map of the collecting sites is shown in Figure 4.2. Pond 100 was located near the town of Churchill and was extremely eutrophic. Pond 73 was situated on the tundra and was the only tundra pond discovered that contained this species. This pond also contained morph B of D. middendorffiana. The reason for this seems to be the lack of Heterocope in the pond as these two species are found only in the absence of this predator (Hebert and Loaring, 1980).

Ponds 102, 103, 103B, 106 and 108 were all located on the riparian mud flats near the Churchill River. D. pulex was also present in these ponds. Ponds 202, 203 and 204 were found on a rock bluff situated very close to Hudson Bay. The ponds were very small being little more than water-filled spaces between rocks. The water in these ponds was considerably saltier than the water in the other seven ponds due to the sea spray. No D. pulex were present in these ponds.

Samples were collected and shipped to Windsor as described in Chapter II . The animals were stored at 5°C and used for electrophoresis within two weeks of collection. One clone each from ponds 100, 108, 73 and 203 was maintained in the laboratory using the methods described

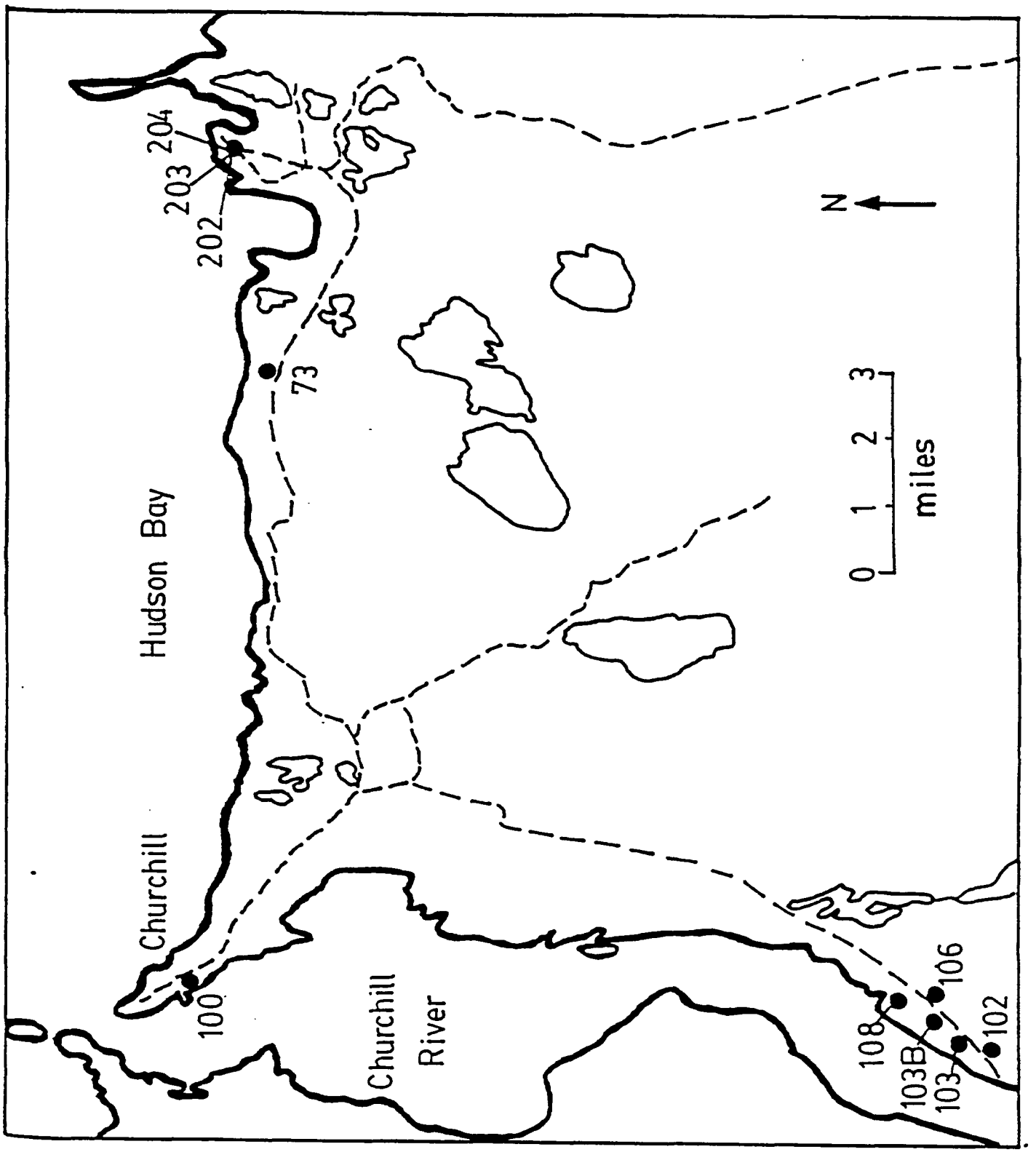


Figure 4.2 Location of Arctic D. magna Populations

in Chapter II. In addition, a sample from each pond was preserved in 95% ethanol.

Fourteen enzymes encoded by 16 loci were analysed electrophoretically according to the methods described in Chapter II. They are alkaline phosphatase (Alk) (2 loci), amylase (Amy), esterase (Est)(2 loci), fumarase (Fum), glucose-6-phosphate dehydrogenase (G6pdh), glutarate oxaloacetate transaminase (Got), hemoglobin (Hb), lactate dehydrogenase (Ldh), leucine aminopeptidase (Lap), malate dehydrogenase (Mdh), phosphoglucoisomerase (Pgi), phosphoglucomutase (Pgm), tetrazolium oxidase (To) and xanthine dehydrogenase (Xdh). Loci were numbered in order of increasing mobility. Alleles were designated slow (S), medium (M), or fast (F) depending on their relative migration rates from the origin. Gene frequencies at each locus were determined by direct count.

The gene frequency data used in the analysis of the 25 populations from Cambridge, England were from Hebert (1975). Eleven loci from nine systems were examined in this study. They are Alk-1, Alk-2, Est-1, Est-2, G6pdh, Hb, Lap, Ldh, Mdh, To, and Xdh. The methods used by Hebert were essentially the same as those used in the present study.

A live sample was obtained from the Hatley Hill pond in the summer of 1977. The population in this pond was polymorphic for two of the three Mdh alleles and all three of the Est-1 alleles found in England. Two clones from this sample were maintained in the laboratory. They were

designated the SF and MF clones on the basis of their different genotypes at the Est-1 locus. These two clones were analysed at all 16 loci and were used in all subsequent laboratory studies involving English animals.

Statistical Analysis

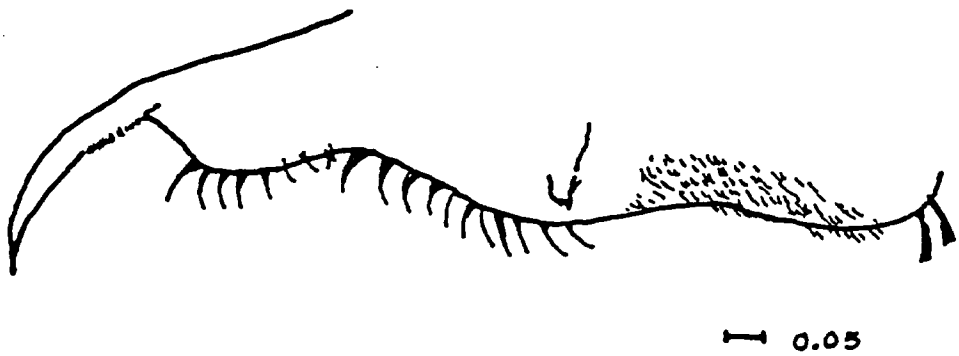
The average heterozygosity per individual and the proportion of polymorphic loci were calculated for each of the 25 English and 10 Arctic populations. Average heterozygosity is given by $\underline{H}=\sum \underline{h}_L/\underline{r}$ where $\underline{h}_L=1-\sum \underline{x}^2_{Li}$. 'L' is the $\underline{L}^{th}$ locus, $\underline{r}$ is the number of loci and $\underline{x}_{Li}$ is the frequency of the $\underline{i}^{th}$ allele at the $\underline{L}^{th}$ locus (Nei, 1975). Nei's statistics $\underline{I}$, genetic similarity, and $\underline{D}$, genetic distance, were calculated for every pair of populations. These parameters were discussed in more detail in Chapter III. The calculations were done using only the gene frequencies at the 11 loci studied by Hebert (1975). From these values the average similarity and distance was calculated for Arctic pairs, English pairs and mixed pairs of populations.

In order to incorporate the additional information provided by the English laboratory clones, $\underline{I}$ and $\underline{D}$ were calculated for each pair of clones SF, MF, 100 and 203 using all 16 loci.

Brooks (1957) has suggested that there may be some morphological differences between European and North American *D. magna*. Drawings of the pattern of anal teeth in

European D. magna differ from what he has observed in North American individuals. Figure 4.3 shows the postabdomen of a North American specimen. Note the small spines inbetween the two large sets of spines. Brooks indicates that drawings of European Daphnia do not show the small spines. He suggested that this character may be useful in distinguishing D. magna from Europe and North America. To determine if this was the case the total number of spines on both sides of the postabdomen was counted in 100 animals collected directly from eight of the Arctic ponds. One hundred animals were also counted from clones SF, MF, 100 and 203 as well as the hybrid clones H2 and H4 (see Chapter V). An analysis of variance was carried out to determine if there were significant differences in spine number among the various populations and clones. Duncan's multiple range test was used to rank populations in terms of spine number and to group populations whose mean spine numbers were not significantly different from one another.

Figure 4.3 Anal Spines of Female D.magna



from Brooks, 1957

RESULTS

Allozyme Patterns

Figure 4.4 shows the allozyme banding patterns for five loci. FUM, XDH, PGM, AMY, LAP, GOT, TO, ALK-2, G6PDH, PGI and EST-2 were all represented by a single band of activity in homozygotes. PGI and ALK-2 heterozygotes had three bands indicating that these enzymes are dimers. HB and MDH homozygotes both had a two-banded pattern. Hebert (1972) has shown that, in the case of MDH, both bands are the product of one locus. Heterozygotes have six bands; four homozygote bands and two hybrid bands indicating that MDH is a dimer as well. Since no variation has been detected at the Hb locus in Daphnia it is not yet known if the two bands are produced by one locus or two.

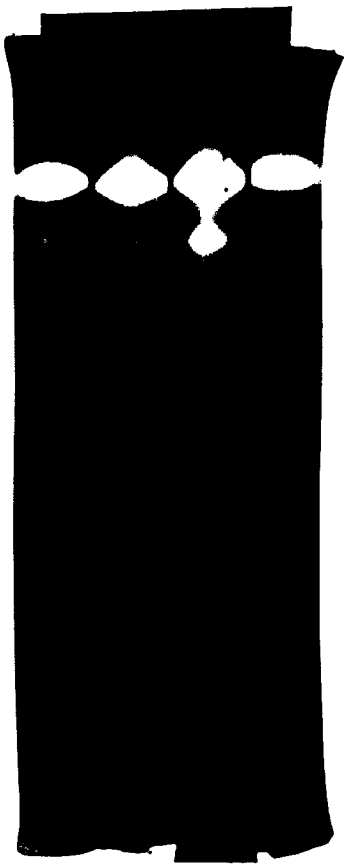
LDH had three bands of activity with the slowest band being most intense. A nine-banded LDH pattern was found in D. pulex in addition to a three-banded pattern similar to the one seen in D. magna (Chapter III). The bands were in three groups of three, each group corresponding to one of the homozygote bands. Since all three bands were affected similarly, it was assumed that the LDH pattern was coded for by one locus and that the protein was a dimer. The multiple bands in the homozygote may be caused by the binding of charged molecules such as NAD as in the case of

Figure 4.4 Allozyme Banding Patterns in D. magna

Leucine
Aminopeptidase

FF SF SS

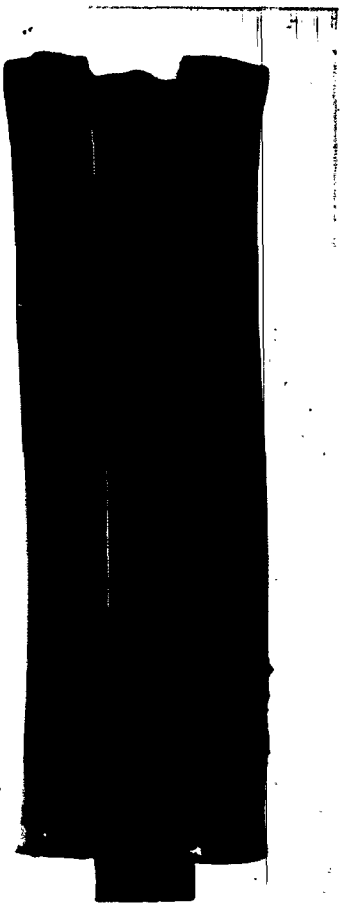
Amylase



Only the top locus was scored

Figure 4.4 Continued

Malate
Dehydrogenase



MM

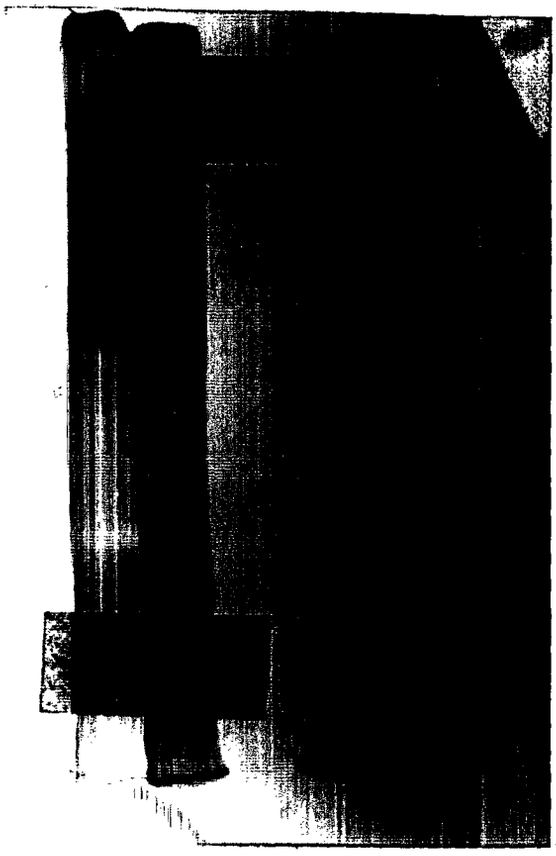
The white band near
the bottom of the gel
is Tetrazolium oxidase

Glucose - 6 - phosphate
Dehydrogenase

Slow band is D. magna
Fast band is D. pulex

Figure 4.4 Continued

Fumarase



alcohol dehydrogenase in Drosophila (Jacobsen, 1968).

EST-1 homozygotes in English animals had a simple two-banded pattern while heterozygotes had four bands. The Arctic pattern was very complex with several bands of various intensities. The pattern was identical in all Arctic animals but the relationship between this pattern and the English pattern could not be determined. These changes were not simply the result of allele substitions at this enzyme locus. More extensive, qualitative changes have developed between the two strains. For the present the Arctic pattern has been labelled the "Arctic allele".

ALK-2 is a single wide band of activity in both groups but some sub-banding is occasionally seen in Arctic animals. These sub-bands do not seem to be genetically determined.

Genetic Variation in English and Arctic Populations

The Arctic populations were genetically homogeneous, being invariant at 15 of the 16 loci. Eight of the populations were also monomorphic for the other locus, Mdh, but two populations (203 and 204) possessed a second allele in very low frequency (Table 4.1). The English populations were more variable, displaying variation at three of the 11 loci. The gene frequencies for these three loci are shown in Table 4.1. The other eight loci were invariant in all the English populations.

Table 4.2 shows the average amount of heterozygosity

Table 4.1 Gene Frequencies at Five Variable Loci in *D. magna* Populations From England and Arctic Canada.*

Populations	Est-1				
	n	S	M	F	A
1. Longstowe Moat**	120	1.00			
2. Longstowe Field-I	96	1.00			
3. Bourn	48	0.12	0.77	0.11	
4. Toft	48	0.04	0.45	0.51	
5. Girton	72	0.33	0.67		
6. Oakington	143		0.45	0.55	
7. Landbeach	128	0.26	0.01	0.73	
8. Goose Hall-I	96		1.00		
9. Goose Hall-II	120		0.99	0.01	
10.Goose Hall-III	96		0.94	0.06	
11.Upware-II	119	0.16	0.38	0.46	
12.Upware-I	144		0.58	0.42	
13.Upware Farm	144	0.21	0.28	0.51	
14.Before Wicken	140	0.72	0.11	0.17	
15.Hatley Hill	120	0.34	0.52	0.14	
16.Hatley St. George	48		1.00		
17.Longstowe Field-II	96	1.00			
18.Longstowe	48	1.00			
19.Past Bourn	120	1.00			
20.Harlton	120		0.98	0.02	
21.Cottenham	72		1.00		
22.Upware Farm-II	142	0.14	0.27	0.59	
23.Wicken	106	0.59	0.39	0.02	
24.Moulton	96			1.00	
25.Audley End	120	0.10	0.50	0.40	
73	48				1.00
100	48				1.00
102	48				1.00
103	48				1.00
103B	48				1.00
106	48				1.00
108	48				1.00
202	48				1.00
203	48				1.00
204	48				1.00

Table 4.1 Continued.

Population	Mdh				G6pdh		
	n	S	M	F	n	S	F
1. Longstowe Moat	96	0.08	0.90	0.02	48	1.00	
2. Longstowe Field-I	100	0.35	0.65		48	1.00	
3. Bourn	114	0.15	0.33	0.52	48	1.00	
4. Toft	96		0.92	0.08	48	1.00	
5. Girton	72		0.76	0.24	48	1.00	
6. Oakington	144		0.41	0.59	48	1.00	
7. Landbeach	315	0.18		0.82	48	1.00	
8. Goose Hall-I	122		0.48	0.52	48	1.00	
9. Goose Hall-II	95		0.53	0.47	48	1.00	
10.Goose Hall-III	165		0.30	0.70	48	1.00	
11.Upware-II	217	0.09	0.74	0.17	48	1.00	
12.Upware-I	96		1.00		48	1.00	
13.Upware Farm	144		0.54	0.46	48	1.00	
14.Before Wicken	120		0.54	0.46	48	1.00	
15.Hatley Hill	88		0.50	0.50	48	1.00	
16.Hatley St. George	48		1.00		48	1.00	
17.Longstowe Field	96	0.51	0.49		48	1.00	
18.Longstowe	72	0.08	0.92		48	1.00	
19.Past Bourn	96	0.50	0.50		48	1.00	
20.Harlton	188		0.55	0.45	48	1.00	
21.Cottenham	72		1.00		48	1.00	
22.Upware Farm	140		0.66	0.34	48	1.00	
23.Wicken	132		0.40	0.60	48	1.00	
24.Moulton	100		0.50	0.50	48	1.00	
25.Audley End	72		0.50	0.50	48	1.00	
73	96		1.00		48		1.00
100	48		1.00		48		1.00
102	96		1.00		48		1.00
103	48		1.00		48		1.00
103B	48		1.00		48		1.00
106	96		1.00		48		1.00
108	48		1.00		72		1.00
202	48		1.00		48		1.00
203	192	0.02	0.98		48		1.00
204	184	0.03	0.97		48		1.00

Table 4.1 Continued.

Populations	Alk-2				Lap		
	n	S	M	F	n	S	F
1. Longstowe Moat	48			1.00	48		1.00
2. Longstowe Field-I	48			1.00	48		1.00
3. Bourn	48			1.00	48		1.00
4. Toft	48			1.00	48		1.00
5. Girton	48			1.00	48		1.00
6. Oakington	48			1.00	48		1.00
7. Landbeach	48			1.00	48		1.00
8. Goose Hall-I	48			1.00	48		1.00
9. Goose Hall-II	48			1.00	48		1.00
10.Goose Hall-III	48			1.00	48		1.00
11.Upware-II	48			1.00	48		1.00
12.Upware-I	48			1.00	48		1.00
13.Upware Farm	48			1.00	48		1.00
14.Before Wicken	48			1.00	48		1.00
15.Hatley Hill	48			1.00	48		1.00
16.Hatley St. George	48			1.00	48		1.00
17.Longstowe Field-II	48			1.00	48		1.00
18.Longstowe	48			1.00	48		1.00
19.Past Bourn	48			1.00	48		1.00
20.Harlton	48			1.00	48		1.00
21.Cottenham	48			1.00	48		1.00
22.Upware Farm-II	48			1.00	48		1.00
23.Wicken	48			1.00	48		1.00
24.Moulton	48		0.50	0.50	48		1.00
25.Audley End	48			1.00	48		1.00
73	72	1.00			72	1.00	
100	48	1.00			48	1.00	
102	72	1.00			48	1.00	
103	48	1.00			48	1.00	
103B	72	1.00			48	1.00	
106	48	1.00			72	1.00	
108	72	1.00			72	1.00	
202	48	1.00			48	1.00	
203	48	1.00			48	1.00	
204	48	1.00			48	1.00	

* Alk-1, Est-2, To, Xdh, Ldh and Hb were monomorphic for the same allele in all 35 populations.

** Data for English populations (1 to 25) is from Hebert, 1975.

Table 4.2 Summary of Genetic Variation in D. magna Populations.

1 to 25 are English
73 to 204 are Arctic

Population	Number of Loci	Heterozygosity per Individual	Proportion of Polymorphic Loci
English			
1	11	0.0995	0.182
2	11	0.00	0.00
3	11	0.0167	0.091
4	11	0.0454	0.091
5	11	0.0414	0.091
6	11	0.014	0.091
7	11	0.0455	0.091
8	11	0.089	0.182
9	11	0.0621	0.182
10	11	0.0761	0.182
11	11	0.0833	0.182
12	11	0.089	0.182
13	11	0.0632	0.182
14	11	0.0454	0.091
15	11	0.0471	0.182
16	11	0.1288	0.182
17	11	0.00	0.00
18	11	0.094	0.182
19	11	0.0443	0.091
20	11	0.1013	0.182
21	11	0.0917	0.182
22	11	0.0852	0.182
23	11	0.089	0.182
24	11	0.091	0.182
25	11	0.0982	0.182
Mean	11	0.0656	0.146

Table 4.2 Continued

Population	Number of Loci	Heterozygosity per Individual	Proportion of Polymorphic Loci
Arctic			
73	16	0.00	0.00
100	16	0.00	0.00
102	16	0.00	0.00
103	16	0.00	0.00
103B	16	0.00	0.00
106	16	0.00	0.00
108	16	0.00	0.00
202	16	0.00	0.00
203	16	0.0036	0.0625
204	16	0.0025	0.0625
Mean	16	0.0006	0.0125

per individual and the proportion of polymorphic loci for each of the 35 populations. The mean heterozygosity in English populations was 6.56% with values ranging from zero to 12.88%. On average, 14.55% of the loci were polymorphic. Sixteen populations were polymorphic at two loci, seven were polymorphic at one locus while two populations were monomorphic at all 11 loci. Mean heterozygosity in Arctic populations was only 0.06% with values ranging from zero to 0.36%. The mean percentage of polymorphic loci was 1.25% however only two populations actually had any polymorphic loci (Table 4.1).

When the two groups of populations were compared to each other, three categories of loci became apparent. The first consisted of loci that were monomorphic for the same allele in both England and the Arctic. These loci included Ldh, To, Xdh, Hb, Alk-1 and Est-2. The second category was comprised of loci at which gene substitutions had occurred and included G6pdh, Lap, Alk-2 and Est-1. G6pdh was monomorphic within groups but differed between groups. The Arctic populations were monomorphic for the S allele at the Lap locus while Hebert's original analysis showed the English populations to be monomorphic for a faster allele. Subsequent analysis of laboratory clones, however, has shown that English populations possess at least three alleles. This will be discussed in more detail further on.

There were three alleles at the Alk-2 locus; the Arctic populations were monomorphic for the S allele whereas

the English populations had two alleles, M and F. English populations had three Est-1 alleles with a fourth being found only in the Arctic (the so-called "Arctic allele" mentioned earlier).

The third category of loci included those that were polymorphic for the same alleles in both groups of populations. Mdh fit into this category as both English and Arctic populations were polymorphic for the S and M alleles. The English also had an additional allele, F, not in the Arctic.

The genotypes of clones SF, MF, 100 and 203 at eight variable loci are listed in Table 4.3. The clonal comparisons extended analysis at five additional loci. Fum and Amy were found to be monomorphic for the same allele in all clones. Both English clones were found to be SF heterozygotes at the Pgi locus while Arctic clones were FF homozygotes.

Two more cases of gene substitution between England and the Arctic were also discovered, namely Got and Pgm. English clones possessed the Got-S allele and the Arctic clones had the F allele. The Arctic had the F allele at the Pgm locus, the English clones had the S allele.

The Lap locus, originally thought to be invariant in English populations, was variable for at least three alleles. The SF clone was homozygous for the F allele and MF clone was heterozygous for the M and Nul alleles (see Chapter V).

Table 4.3 Genotypes of Clones SF, MF, 100 and 203 at Eight Variable Loci.

Clone	Mdh	Est-1	Alk-2	Lap	Pgm	Got	G6pdh	Pgi
SF	MF	SF	MM	FF	SS	SS	SS	MF
MF	MF	MF	MM	MNul	SS	SS	SS	MF
100	MM	A	SS	SS	FF	FF	FF	FF
203	SM	A	SS	SS	FF	FF	FF	FF

Arctic clones were homozygous for the S allele. A summary of the allelic arrays present in the two groups is shown in Table 4.4.

These results suggest that the level of genetic variability in the Hatley Hill population, and most likely other English populations is higher than was originally thought.

From the above it can be seen that considerable diversity exists between English and Arctic Daphnia populations. Calculation of the genetic similarity and distance between populations further stressed this point. The matrix of similarities and distances is shown in Table 4.5. Mean similarities and distances for the different groups are listed in Table 4.7.

Populations within groups were quite similar to one another. When rounded to three decimal places, the similarity between all Arctic populations was 1.000. The mean genetic similarity between English populations was 0.946 (0.846-1.00). In contrast to this, the mean similarity between English and Arctic populations was 0.62 (0.564-0.651) and the mean genetic distance was o.479 (0.429-0.574). Therefore, while populations from the same area differed at less than 15% of their loci, any two populations from different areas were likely to differ at about 48% of their loci.

Table 4.6 shows I and D values for each pair of laboratory clones. Mean similarity between English and Arc-

Table 4.4 Alleles Found at Eight Loci in Populations From England and Arctic Canada*

Locus	English	Arctic
Mdh	S, M, F	S, M
Pgi	S, F	F
Est-1	S, M, F	A
Lap	M, F, Nul	S
Alk-2	M, F	S
Pgm	S	F
G6pdh	S	F
Got	S	F

* All populations were monomorphic for the same alleles at Ldh, Xdh, Fum, Amy, To, Hb, Est-2 and Alk-1.

Table 4.5 Genetic Similarity (above diagonal) and Genetic Distance (below diagonal) Between Populations of D. magna From England and Arctic Canada

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	-	.994	.907	.934	.954	.901	.88	.886	.891	.873	.947	.928	.939
2	.006	-	.913	.924	.948	.901	.894	.885	.889	.875	.944	.917	.936
3	.098	.091	-	.959	.983	.983	.942	.994	.994	.995	.971	.957	.976
4	.068	.079	.042	-	.978	.974	.917	.954	.959	.942	.997	.998	.983
5	.047	.054	.017	.022	-	.965	.906	.982	.985	.971	.983	.98	.947
6	.104	.104	.017	.026	.035	-	.973	.97	.97	.976	.983	.965	.995
7	.128	.112	.059	.087	.098	.027	-	.907	.905	.927	.941	.895	.972
8	.121	.122	.006	.047	.018	.03	.097	-	1.0	.997	.96	.957	.96
9	.116	.118	.006	.048	.015	.03	.1		-	.995	.964	.963	.961
10	.136	.133	.005	.06	.03	.025	.076	.003	.005	-	.952	.941	.961
11	.054	.058	.029	.003	.017	.017	.06	.041	.037	.049	-	.992	.993
12	.074	.087	.044	.002	.02	.036	.111	.043	.038	.061	.008	-	.973
13	.063	.066	.024	.017	.026	.005	.029	.041	.04	.04	.007	.028	-
14	.021	.023	.044	.049	.03	.041	.05	.068	.066	.067	.031	.061	.02
15	.055	.056	.008	.029	.009	.015	.051	.018	.017	.018	.017	.034	.011
16	.097	.11	.039	.027	.015	.063	.167	.025	.02	.047	.034	.016	.06
17	.017	.002	.092	.092	.064	.109	.106	.128	.125	.136	.067	.102	.073
18	.000	.008	.1	.068	.048	.106	.131	.123	.117	.139	.055	.074	.065
19	.016	.002	.092	.091	.063	.108	.107	.128	.124	.136	.066	.101	.072
20	.113	.116	.007	.039	.014	.03	.1	.001	.000	.006	.035	.035	.038
21	.097	.11	.039	.027	.015	.063	.167	.025	.02	.047	.034	.016	.06
22	.065	.07	.033	.009	.028	.009	.04	.048	.045	.05	.004	.018	.002
23	.044	.043	.021	.055	.02	.032	.05	.036	.037	.033	.035	.063	.022
24	.149	.152	.103	.066	.115	.047	.052	.13	.128	.121	.059	.085	.044
25	.08	.082	.011	.018	.019	.003	.038	.02	.02	.019	.01	.025	.004
73	.458	.482	.506	.431	.449	.493	.574	.506	.497	.533	.44	.429	.467
100	.458	.482	.506	.431	.449	.493	.574	.506	.497	.533	.44	.429	.467
102	.458	.482	.506	.431	.449	.493	.574	.506	.497	.533	.44	.429	.467
203	.459	.481	.504	.433	.45	.493	.57	.506	.497	.531	.441	.431	.466
103B	.458	.482	.506	.431	.449	.493	.574	.506	.497	.533	.44	.429	.467
106	.458	.482	.506	.431	.449	.493	.574	.506	.497	.531	.44	.431	.467
108	.458	.482	.506	.431	.449	.493	.574	.506	.497	.531	.44	.431	.467
202	.458	.482	.506	.431	.449	.493	.574	.506	.497	.531	.44	.431	.467
103	.458	.482	.506	.431	.449	.493	.574	.506	.497	.531	.44	.431	.467
204	.459	.481	.505	.432	.449	.493	.571	.506	.497	.532	.441	.43	.466

Table 4.5 Continued

	14	15	16	17	18	19	20	21	22	23	24	25	73
1	.979	.947	.908	.984	1.00	.984	.893	.908	.937	.957	.862	.923	.633
2	.978	.946	.896	.998	.993	.997	.891	.896	.932	.958	.859	.922	.617
3	.957	.992	.962	.912	.905	.912	.993	.962	.968	.98	.903	.99	.603
4	.952	.971	.973	.912	.934	.913	.962	.973	.991	.947	.936	.982	.65
5	.971	.991	.985	.938	.954	.939	.986	.985	.972	.98	.891	.981	.638
6	.96	.985	.939	.897	.899	.897	.971	.939	.991	.968	.954	.997	.611
7	.952	.951	.846	.899	.877	.899	.904	.846	.961	.951	.95	.963	.564
8	.935	.982	.975	.88	.884	.88	.999	.975	.953	.965	.878	.98	.603
9	.936	.983	.98	.882	.89	.883	1.0	.98	.956	.964	.88	.981	.608
10	.935	.982	.954	.872	.87	.873	.994	.954	.951	.967	.886	.981	.587
11	.97	.983	.967	.935	.947	.936	.966	.967	.996	.966	.943	.99	.644
12	.941	.966	.984	.903	.929	.904	.965	.984	.982	.939	.919	.975	.651
13	.98	.989	.942	.93	.937	.93	.962	.942	.998	.978	.956	.996	.627
14	-	.984	.917	.971	.978	.972	.937	.917	.972	.992	.914	.97	.622
15	.016	-	.96	.94	.945	.94	.983	.96	.982	.994	.918	.994	.623
16	.086	.041	-	.883	.908	.884	.981	1.0	.947	.932	.858	.957	.636
17	.029	.062	.124	-	.983	1.00	.884	.883	.924	.954	.853	.916	.604
18	.023	.056	.1	.017	-	.984	.892	.908	.936	.955	.86	.921	.633
19	.029	.062	.123	.0	.017	-	.885	.884	.925	.954	.854	.916	.605
20	.065	.017	.019	.123	.114	.122	-	.981	.958	.964	.882	.981	.61
21	.086	.041	.0	.124	.1	.123	.019	-	.947	.932	.858	.957	.636
22	.029	.018	.054	.079	.066	.078	.043	.054	-	.966	.959	.993	.635
23	.008	.006	.07	.047	.046	.047	.037	.07	.034	-	.901	.979	.61
24	.09	.085	.153	.158	.151	.158	.126	.153	.041	.104	-	.944	.62
25	.03	.066	.044	.088	.082	.088	.019	.044	.007	.021	.058	-	.622
73	.475	.474	.452	.504	.457	.503	.494	.452	.454	.495	.478	.474	-
100	.475	.474	.452	.504	.457	.503	.494	.452	.454	.495	.478	.474	.0
102	.475	.474	.452	.504	.457	.503	.494	.452	.454	.495	.478	.474	.0
203	.475	.473	.454	.502	.458	.5	.493	.454	.454	.494	.478	.474	.0
103B	.475	.474	.452	.504	.457	.503	.493	.452	.454	.495	.478	.474	.0
106	.475	.474	.452	.504	.457	.503	.493	.452	.454	.495	.478	.474	.0
108	.475	.474	.452	.504	.457	.503	.493	.452	.454	.495	.478	.474	.0
202	.475	.474	.452	.504	.457	.503	.493	.452	.454	.495	.478	.474	.0
103	.475	.474	.452	.504	.457	.503	.493	.452	.454	,495	.478	.474	.0
204	.475	.473	.453	.503	.457	.501	.493	.453	.454	.494	.478	.474	.0

Table 4.5 Continued

	100	102	203	103B	106	108	202	103	204
1	.633	.633	.632	.633	.633	.633	.633	.633	.632
2	.617	.617	.618	.617	.617	.617	.617	.617	.618
3	.603	.603	.604	.603	.603	.603	.603	.603	.604
4	.65	.65	.649	.65	.65	.65	.65	.65	.649
5	.638	.638	.638	.638	.638	.638	.638	.638	.638
6	.611	.611	.611	.611	.611	.611	.611	.611	.611
7	.564	.564	.565	.564	.564	.564	.564	.564	.565
8	.603	.603	.603	.603	.603	.603	.603	.603	.603
9	.608	.608	.608	.608	.608	.608	.608	.608	.608
10	.587	.587	.588	.587	.587	.587	.587	.587	.588
11	.644	.644	.644	.644	.644	.644	.644	.644	.644
12	.651	.651	.65	.651	.651	.651	.651	.651	.65
13	.627	.627	.627	.627	.627	.627	.627	.627	.627
14	.622	.622	.622	.622	.622	.622	.622	.622	.622
15	.623	.623	.623	.623	.623	.623	.623	.623	.623
16	.636	.636	.635	.636	.636	.636	.636	.636	.636
17	.604	.604	.606	.604	.604	.604	.604	.604	.605
18	.633	.633	.633	.633	.633	.633	.633	.633	.633
19	.605	.605	.606	.605	.605	.605	.605	.605	.606
20	.61	.61	.611	.61	.61	.61	.61	.61	.611
21	.636	.636	.635	.636	.636	.636	.636	.636	.636
22	.635	.635	.635	.635	.635	.635	.635	.635	.635
23	.61	.61	.61	.61	.61	.61	.61	.61	.61
24	.62	.62	.62	.62	.62	.62	.62	.62	.62
25	.622	.622	.622	.622	.622	.622	.622	.622	.622
73	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
100	-	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
102	.0	-	1.0	1.0	1.0	1.0	1.0	1.0	1.0
203	.0	.0	-	1.0	1.0	1.0	1.0	1.0	1.0
103B	.0	.0	.0	-	1.0	1.0	1.0	1.0	1.0
106	.0	.0	.0	.0	-	1.0	1.0	1.0	1.0
108	.0	.0	.0	.0	.0	-	1.0	1.0	1.0
202	.0	.0	.0	.0	.0	.0	-	1.0	1.0
103	.0	.0	.0	.0	.0	.0	.0	-	1.0
204	.0	.0	.0	.0	.0	.0	.0	.0	-

Table 4.6 Genetic Similarity (above diagonal) and Genetic Distance (below diagonal) Between English and Arctic Laboratory Clones.

Clone	SF	MF	100	203
SF	-	0.93	0.5909	0.591
MF	0.0726	-	0.6013	0.6014
100	0.5261	0.5086	-	0.9999
203	0.526	0.5084	0.0001	-

Table 4.7 Mean Genetic Similarity and Distance Between English and Arctic Populations and Clones.

	English	Arctic	English vs Arctic
	Genetic Similarity (I)		
Populations	0.9495 (0.8462-1.00)	0.99998 (0.99992-1.00)	0.6196 (0.5635-0.6509)
Clones	0.93	0.99995	0.5962 (0.5909-0.6014)
	Genetic Distance (D)		
Populations	0.058 (0.00-0.1669)	0.00002 (0.00008-0.00)	0.479 (0.4293-0.5735)
Clones	0.0726	0.00005	0.5173 (0.5084-0.5261)

tic clones was 0.596. When the Hatley Hill population (the source of clones SF and MF) was compared to the Arctic populations using only 11 loci, the mean similarity was 0.623. The standard errors of these estimates were very large due to the small number of loci that were studied. As a result, the values of I for the clones and for the populations were not significantly different from one another.

Morphological Variation

The data on spine numbers was checked for normality and tests for skewness and kurtosis were found to be insignificant ($t=0.707$, $p>0.05$; $t=0.142$, $p>0.05$). Homoscedascity was tested using the F_{max} test and found to be slightly significant even after the data were transformed using a natural log transformation ($F=1.5$, $p>0.05$). According to Sokal and Rohlf (1969) this is not too serious provided the comparisons involve more than one degree of freedom. It was necessary to perform the ANOVA as all ranking procedures use information from this analysis.

The ANOVA was highly significant($F=78.16$, $p=0.0001$) so there is no doubt that there are differences between populations. There may be some inaccuracy in ranking the populations, particularly those whose mean spine numbers were very close.

The mean number of postabdominal spines and its var-

iance are listed for each population and clone in Table 4.8. Clones were found to exhibit as much variation in spine number as did populations suggesting that the environmental component of variance is large and that genetic variation in spine numbers among clones in ponds is small.

The results of the Duncan's multiple range test (Table 4.8) show significant differences in mean spine number among several populations. Three groups of Arctic populations can be recognized. The first group is population 73, the tundra pond, with a very high spine number of 34.94. The next group is the ponds on the mudflats, 103B, 102 and 108. Included in this group is pond 100. Note however that pond 100 and 108 were significantly different from one another. The last group is the rock bluff ponds, 203, 204 and 202, with relatively low spine numbers. English clones MF and SF fell between the last two groups of Arctic ponds.

The mean spine number of clone 203 and clone 100 were much higher than that of populations 203 and 100 respectively. Clone 100 had the highest mean spine number of all the samples counted. This may indicate that growing the clones under laboratory conditions as opposed to natural pond conditions affects spine number.

The mean spine number of hybrid clone H2 (35.48) was closer to the value of the Arctic parent, 100 (36.61), than to the English parent, SF (30.94). The midparent value was 33.74 suggesting that there is some dominance

for high spine number. Unfortunately, F_2 offspring were not available for analysis.

Hybrid clone, H4, appears to show the same pattern as its mean spine number, 33.41, was higher than that of its English parent, SF (30.94). Unfortunately laboratory clone 108 was not available at the time of this analysis, however the value for H4 was higher than the value for population 108 (33.24). The same result was obtained for H2 which had a higher mean spine number than population 100 (34.23).

Table 4.8 Mean Spine Number and Variance for the *D. magna* Populations and Clones.

Populations with the same letter were not significantly different according to the Duncan's multiple range test. Mean Square=8.908, D.F.=1386, alpha=0.05.

Group	Population	Mean	Variance
A	clone 100	36.61	13.53
A B	73	35.94	8.78
A B	clone 203	35.79	13.93
B	H2	35.48	8.66
C	100	34.23	12.20
C D	103B	34.04	8.24
C D	102	33.95	8.73
C D	H4	33.41	9.29
D	108	33.24	7.94
E	clone MF	31.97	7.91
F	clone SF	30.94	4.99
G	203	29.90	7.53
G	204	29.72	5.74
H	202	27.94	7.25

DISCUSSION

The results of this study have shown that the Daphnia populations within each group have remained quite similar to one another. The Arctic populations were virtually identical; all but two of the ten populations were composed of a single genotype based on the 16 loci that were analysed. If rounded to three decimal places, the genetic similarity between all pairs of Arctic populations was 1.000. Similarity values for pairs of English populations ranged from 0.846 to 1.00 with a mean of 0.946. Most of the differences between these populations resulted from variation in gene frequencies at polymorphic loci. Only one population contained an allele that was unique to it alone (Alk-2 F allele in Moulton). The similarity values for English populations are similar in magnitude to values calculated for conspecific populations of other organisms. For example, Richmond (1972) compared populations within the same semi-species of Drosophila paulistorum and found an average similarity value of 0.884.

In contrast to the within-group similarity of Daphnia populations, considerable genetic divergence has occurred between the English and Arctic metapopulations. Fifty percent of the loci examined had alleles that were unique to each metapopulation. The mean similarity for inter-group comparisons was 0.62 which falls in the range of

sibling species of Drosophila. Recall, however that sibling species show complete reproductive isolation from one another while crosses between the English and Arctic D. magna produced viable offspring (Chapter V). To date, such low similarity values have never been found between populations within the same species. Low genetic similarity is almost always associated with reproductive isolation. There are exceptions, but in these cases reproductive isolation has evolved without much genetic divergence. There does not seem to be any other case where reproductive isolation has not evolved even after the accumulation of so many genetic differences.

Despite the extensive genetic change detectable at the biochemical level, individuals from the two areas were nearly indistinguishable morphologically. Variation among populations was found in one morphological trait (spine number) but much of this variation appeared to be non-genetic. In most cases, clones, whose members are genetically identical, possessed as much variation in spine number as did populations. The fact that mean spine number of the English clones fell between that of the two groups of Arctic populations mentioned earlier shows that there is no clear distinction between English and Arctic Daphnia with respect to spine number.

The morphological uniformity of species such as Daphnia has been attributed to high vagility which supposedly

results in panmixia across large geographic ranges. In other words, gene flow has retarded evolutionary change. But, the extensive genetic differentiation between English and Arctic Daphnia shows that this is not the case at all. The message is clear; morphological change does not accompany genetic divergence. In fact, there are many examples in the literature in which the degree of morphological differentiation does not parallel the degree of biochemical variation. One of the best examples is the case of sibling species of Drosophila which, although extremely similar morphologically, are quite different allozymically. Genetic similarity values for several sibling species pairs range from 0.466 to 0.788 with an average of about 0.489 (Ryman et al., 1979). Similarly, Nicklas and Hoffman (1979) found extensive biochemical variation ($\underline{I}$=0.468) between two species of polychaetes (Glycera dibranchiata and G. americana) although they have remained very similar phenotypically.

Nixon and Taylor (1977) studied 22 populations of the planarian Polycelis coronata from various locations in Washington state. These organisms live in streams between which gene flow is relatively restricted. Nixon and Taylor calculated a "coefficient of association" based on similarity in protein banding patterns. The average coefficient of association between two populations was 0.51 with values ranging from 0.17 to 0.95 indicating extensive allozyme divergence between populations. There was, how-

ever, very little phenotypic variation among them.

There are also cases where the opposite is true, that is, populations or species have remained similar biochemically but have diverged morphologically. Some examples of this are; a comparison of two species of minnow, Hesperoleucus symmetricus and Lavinia exilicauda, in different genera but with a similarity of 0.948 (Avise et al,. 1975); a comparison of five species of pupfish with similarity values ranging from 0.808 to 0.968 (Turner, 1974); and a comparison of several subspecies of the genus Peromyscus whose similarity values were all greater than 0.945 (Zimmerman et al, 1979). In all these examples the species could easily be distinguished from one another on the basis of morphology.

Zimmerman et al. (1979) have suggested that, in Peromyscus at least, populations respond to different selection pressures through changes in morphology and behaviour while proteins involved in biochemical functions remain fairly conservative. Turner (1974) suggested a similar mechanism for pupfish in which biochemical regions of the genome evolve slowly while the region controlling morphology tends to evolve much more rapidly.

Most cases where morphological changes preceded biochemical changes seemed to involve vertebrates. Evolutionary changes in invertebrates, on the other hand, (such as Drosophila, Planaria, Polychaetes and Daphnia) tended to involve biochemical and physiological changes with ap-

parent phenotypic stability. It has been suggested by many (Zimmerman et al. 1979; Turner, 1974; Nixon and Taylor, 1977; Mayr, 1963 and Mitton, 1976) that the phenotype in such organisms is canalized against change. Lerner (1954) has termed this "genetic homeostasis" or "the property of the population to equilibrate its genetic composition and to resist sudden changes". The overall phenotype of an organism is the product of the action and interaction of many genes. Mayr suggested that there can be many ways to achieve the same result so, although genetic changes are occurring, they are not manifested in the visible phenotype. He cites several examples of this, for instance, the two sibling species Drosophila melanogaster and D. simulans have identical scutellar bristle patterns and yet when they are crossed many bristles are missing or poorly developed in the F_1 hybrids (Sturtevant, 1929). Clearly the developmental pathways responsible for the pattern of bristles are different in the two species (as shown by their incompatibility) even though the end result appears to be the same.

The existance of sibling species (which do not normally exchange genes) illustrates that dramatic alterations of the genome can occur without affecting the phenotype. It is quite possible that a similar phenomenon is occurring in Daphnia populations. We know that extensive genetic changes have occurred in the two groups of Daphnia populations examined in the present study as shown by the

high degree of allozyme differentiation. Other evidence of this divergence was also found in a study of some important physiological traits such as the induction of male and ephippial female production. Arctic Daphnia tended to produce ephippia in response to short-day photoperiods, whereas English Daphnia responded more strongly to crowding and decreased food supply (Chapter VI ; Woodrich, 1980). It is, therefore, reasonable to expect that changes are also occurring in the regions of the genome that control morphology. But if the daphnid genome is, in the words of Mayr (1963, p. 281), a "well-integrated gene complex held together by genetic homeostasis" we could expect alterations in polygenic traits (such as those determining the visible phenotype) to be resisted in the face of extensive genetic change.

This same argument could apply to Daphnia pulex as well. The clones described in Chapter III reproduced by obligate parthenogenesis and were, therefore, incapable of exchanging genes, yet the phenotype remained stable. The genotype, however, had diverged considerably. Similarity values between clones ranged from 0.667 to 0.977 ($\bar{I}$=0.869). Again, canalization of the phenotype has prevented visible alterations in overall morphology.

Although considerable genetic variation existed between populations from the Arctic and England, individual populations seemed to possess less variation than that found in other populations of invertebrates. The average

proportion of polymorphic loci, calculated for several invertebrates by Hamrick (1979) was 0.469 (0.175 to 0.587) and the average heterozygosity per individual was 0.135 (0.062 to 0.151). Average heterozygosity per locus per individual in English populations was only about 7% with approximately 15% of the loci being polymorphic. If the Cambridge populations are considered as a whole, the proportion of polymorphic loci was 36% (four of 11 loci) which is not appreciably less than that found in other invertebrates. Hebert (1975) pointed out that, because the Daphnia gene pool is fragmented into isolated demes with reduced variation due to founder effects, a single population is not representative of the species as a whole. He suggested that in order to accurately appraise the level of variability in such a species many populations must be sampled. The results of the analysis of the Arctic populations confirm this. If the Arctic populations were considered separately one would be led to believe that the species had virtually no variability. However, by combining the data from England and the Arctic we find variation at eight out of 16 or 50% of the loci examined. Taking this into consideration it seems that Daphnia magna as a species is extremely variable. Most of the variability, as suggested earlier, is maintained as differences between populations. In the English populations however, heterozygote excesses were often noted at polymorphic loci. Similar observations have been made in populations of the

inbred oat species Avena barbata in California (Clegg and Allard, 1972; Hamrick and Allard, 1972). In some areas populations were monomorphic at all enzyme loci surveyed, while in other areas several loci were polymorphic. The monomorphic populations tended to be in the extreme habitats, in this case, those that were most xeric and most mesic. Different homozygotes were associated with the two extremes. Heterozygote excesses were common in the variable populations found in intermediate habitats. Allard and his coworkers argued (as did Hebert (1975) for the Daphnia populations) that the polymorphisms were maintained in the face of strong inbreeding by some sort of balancing selection or heterosis. They felt that variation was maintained in intermediate habitats because the direction and intensity of selection changed irregularly, tending towards mesic conditions in some years and xeric conditions in other years. The extremes, on the other hand, were slightly more predictable in that a mesic habitat was likely to be that way every year. Therefore, the one genotype best suited to mesic conditions predominated in mesic habitats and the one suited to xeric conditions persisted in xeric habitats.

What are the possible explanations for the homozygosity of Arctic populations as opposed to English populations in the case of Daphnia magna? There does not seem to be a clear pattern in the distribution of variation like

that seen in A. barbata. Of the 25 English populations considered only two contained just one electrophoretically recognizable clone. Most had several clones as a result of the multiple genotypes generated by polymorphic loci. The tendency seemed to be to "hang on" to genetic variability when it existed. Why then, are the Arctic populations so invariant?

The most plausible explanation for the homozygosity seems to be founder effect coupled with inbreeding. Brooks (1957) has suggested that most of North America was recolonized after the last glacial retreat of the Pleistocene by populations that survived in glacial refuges in Central Alaska and the Northwest Territories, which are several thousand miles away. The area surrounding Hudson Bay remained under sea water for a considerable period of time even after the ice receded. Therefore it is very likely that the Churchill populations were established relatively recently and have been founded by only one or a few colonists.

In contrast to Churchill, populations in England are much closer to glacial refuges in northern Europe. Gene flow from these areas to England may have been more frequent allowing the introduction of new variation. As a result, English populations are able to maintain more variation than the Churchill populations.

Heterosis and balancing selection seem to be important mechanisms for the maintenance of genetic varia-

bility in English D. magna so it is reasonable to suppose that these factors would be important in the the Arctic populations as well. If this is the case one would expect that any new variation introduced into these populations would be maintained. It would be very valuable to survey other D. magna populations from North America, particularly those from glacial refuges, to see how the level of genetic variation compares with the Churchill populations. The prediction is that they will possess as much or more variation than the English populations. Populations from glacial refuges in northern Europe would also be of considerable interest as this is the area from which the English were most likely derived.

SUMMARY

Allozyme variation was studied at 16 loci in ten populations of D. magna from Churchill, Manitoba. These populations were found to possess extremely low levels of variability. Heterozygosity per individual was only 0.06%. Only two populations showed any variation, being polymorphic at the Mdh locus, but the rare allele was present at a frequency of only about 0.025.

These results were compared to those obtained by Hebert (1975) for 25 populations from Cambridge, England. Here variability was considerably higher. Mean heterozygosity per individual was 0.066 and on average the proportion of polymorphic loci was 0.146. The paucity of variation in the Arctic was related to the fact that the Churchill populations are so far away from glacial refuges in the Northwest Territories and Alaska. It was suggested that founder effect was responsible for the high level of homozygosity in these populations. Genetic similarity was calculated for each pair of the 10 Arctic and 25 English populations. Within group similarity was high; the mean for Arctic comparisons was 1.000, the mean similarity of English populations was 0.946. In contrast, the mean similarity between Arctic and English populations was only 0.62, the lowest value calculated to date for two populations within the same species.

Analysis of laboratory clones from one English and two Arctic populations revealed gene substitutions at 38% of the loci examined. Such extensive genetic divergence is on the order of that seen in sibling species of Drosophila which show complete reproductive isolation from one another.

Despite the extensive genetic differentiation the two strains remained morphologically very similar. Analysis of abdominal spine number in Arctic and English individuals revealed no distinct differences between them. The maintenance of high morphological similarity in the face of extreme genetic divergence is discussed.

CHAPTER V

INTERSTRAIN HYBRIDS OF *DAPHNIA MAGNA*

CHAPTER V

INTRODUCTION

The occurrence of sibling species in many groups of animals suggests that reproductive isolation can evolve without major morphological divergence however, allozyme studies of sibling species have shown them to be genetically quite different from one another (Drosophila spp, Ayala, 1975; Goniabasis floridensis (snail), Chambers, 1978; Albula vulpes (fish), Shaklee and Tamaru, 1977 and Desmognathus ochrophaeus (salamander), Tilley et al., 1978). Mayr (1963) has suggested that a major reorganization of the gene pool such as that seen in sibling species is a necessary pre-requisite for speciation. As genetic differences accumulate within separated populations they become increasingly incompatible until reproductive isolation is complete. Many people have shown, however, that extensive genetic differentiation is not required for the evolution of reproductive isolation. In fact, changes at a few crucial loci are in some cases all that is necessary to develop reproductive isolation. Oliver (1979) showed that interpopulation hybrids of several Lepidoptera species were considerably less viable than individuals from the parent populations. He suggested this was due to slight modifications of the regulatory portion of the genome. Similarly, Prakash (1972) hybridized indiv-

iduals from five different populations of Drosophila pseudoobscura. Four of the populations were from the North American mainland while one, Bogota, was from the highlands of Columbia in South America. This is the only known location of D. pseudoobscura in S. America and as a result this population was extremely isolated from the main range of the species. Prakash found that F_1 males from crosses between Bogota females and mainland males were sterile indicating partial reproductive isolation between this population and the rest of the species. He suggested that the sterility may be a product of as few as four genes, two on the autosomal chromosomes and two on the X chromosome.

Ryman et al. (1979) showed that a population of the trout, Salmo trutta, in a Swedish mountain lake was actually divided into two subpopulations between which there was no gene flow. Even so, Nei's genetic similarity between the subpopulations was 0.975 indicating that very little genetic differentiation had occurred.

These examples have shown that reproductive isolation may often if not always, evolve independently of changes in the phenotype. As a result, extensive genetic differentiation such as that found between the English and Arctic Daphnia magna populations discussed in Chapter IV may or may not be accompanied by the development of reproductive isolation. In order to determine whether or not there was evidence for isolation, crosses between

English and Arctic D. magna were made in the laboratory. Hybrid offspring were produced indicating that isolation, if it did exist, had not progressed to the point where offspring could no longer be produced.

The next step was to compare the fitness of these hybrids to that of the parent clones that produced them. If the English and Arctic Daphnia were becoming reproductively isolated from one another, one would expect that interstrain hybrids would be less viable or fertile than individuals from either pure strain. On the other hand, if no isolating mechanisms had developed the increased heterozygosity possessed by the hybrids might result in heterosis or hybrid vigor.

Mayr (1963) described several possible mechanisms for increased vigor in offspring produced by crosses between populations. It might result from the fact that alleles from one parent strain are masking deleterious alleles that have become homozygous in the other parent strain and vice versa. Another possibility is overdominance where the heterozygote has greater fitness than either homozygote. Mayr suggested that heterozygous enzyme loci cause greater biochemical versatility, allowing the organism to tolerate a greater range of environmental conditions.

Many people have shown that increased levels of heterozygosity can indeed increase the fitness of populations. There have been several experiments showing that chromo-

somally polymorphic Drosophila populations have higher viability and fertility than monomorphic populations. For example Sved and Ayala (1970) studied two types of Drosophila pseudoobscura populations. One type contained individuals that were homozygous for a wild type second chromosome. The other type of population contained flies that were homozygous for the wild chromosome as well as flies that were heterozygous for the wild type chromosome and a marked second chromosome from an inbred stock which was lethal in the homozygous condition. They found that the marker chromosome was maintained in the polymorphic population at a fairly high frequency and that these populations produced more and larger offspring than the homozygous populations. Sved (1971) and Sperlich and Karlik (1970) did similar experiments and obtained similar results using D. melanogaster. Dobzhansky, Lewontin and Pavlosky (1964) also found that populations of D. pseudoobscura which were heterozygous for chromosome inversions had higher fitness and higher rates of increase than populations homozygous for the inversions. Furthermore, Ayala (1969) showed that populations of this species that were heterozygous for the inversions were superior to monomorphic populations in their ability to compete with D. serrata for limited resources.

Other experiments have shown that a simple increase in heterozygosity is not always advantageous. Zali and Allard (1976) crossed one isogenic strain of barley with

16 other isogenic strains and found that not all hybrids showed evidence of heterosis. The degree of heterosis depended largely on the genetic backgrounds of the lines being used suggesting that an increase in heterozygosity may not be advantageous when the genomes being brought together through the hybridization are not compatible.

In order to determine whether or not interstrain hybrids of D. magna show any evidence of heterosis, competition experiments were carried out between several hybrid clones and their parent clones in conditions of limited food and space.

MATERIALS AND METHODS

Production of English-Arctic Hybrids

English clones SF and MF and Arctic clones 100, 108 and 73 were used in matings set up to produce hybrids. Ephippial females were produced by placing large numbers of juveniles in optimal conditions until they became reproductive and then suddenly reducing their food and the volume of their culture. The English females were kept in 24 hour days at room temperature and the Arctic females were kept in four hour days at 20°C. The production of sexual females is discussed in more detail in Chapter VI. Males were easily obtained from cultures that had been allowed to become very crowded.

Reciprocal matings of all combinations of Arctic by English animals were made. Young females that had eggs in their ovaries and an ephippium in the initial stages of formation were placed with equal numbers of males of the appropriate clone. The two sexes were left together until all ephippia were shed.

Ephippia were collected and kept in artificial pond water until hatching was attempted. Hatching was accomplished by air drying the ephippia on a small piece of cheesecloth for two to seven days, storing them at 5°C for one to three weeks and finally rehydrating them in arti-

ficial pond water. The cheesecloth prevented the ephippia from floating to the surface of the water. Hatching usually occurred within one week of rehydration and after two weeks the process was repeated.

Upon hatching, offspring were isolated and maintained in culture as described in Chapter II. All hybrids were electrophoresed for Pgi and Mdh because the English clones were heterozygous at these loci and thus more than one hybrid genotype was possible. After it was discovered that Lap was also heterozygous in the MF clone (see Results), Lap genotypes were also determined for each hybrid.

Got, Pgm, Alk-2, Est-1 and G6pdh were also analysed electrophoretically in two of the hybrids. All these loci involved substitutions so the hybrids, being heterozygous, were able to provide information about the quaternary structure of these enzymes.

Competition Experiments

Competition experiments involved competing hybrid clones separately with each of their parent clones. Ten one to two day old females of the hybrid clone and ten similar females of one parent clone were placed together in one liter of artificial pond water in a 1½ liter jar. Similarly, ten hybrid females were competed with ten females of the other parent clone. Two replicates of each hybrid-parent combination were set up in 24 hour days at 10°C and at room temperature. Cultures were given 80 mls of the Scen-

edesmus/liver mixture twice weekly.

The experiments were run for 120 days at 10° and for 90 days at room temperature. After that, animals were removed and electrophoresed for either Pgi, Mdh or Lap to determine the frequencies of each clone. Parent clones were homozygous and hybrid clones were heterozygous at the Lap locus. In the case of Pgi and Mdh, the genotypes depended on which parent and which hybrid were being considered (Table 5.1).

If possible, at least forty-eight animals from each jar were typed. If there were not enough adults present the jar was sampled again after the juveniles had grown to electrophoresible size. If both clones were still present the sample was increased.

Results were expressed as the proportion of the parent clone remaining in the jar. After an arcsine transformation of the data, a two-way analysis of variance with temperature and competitor as the main effects was performed.

RESULTS

Mating Behaviour

Both Arctic and English males mated readily with females from different clones. They did not seem to be attracted specifically to ephippial females, however, as they were often observed mating with asexual and non-reproductive females even when sexual females were present. Sometimes two males were seen mating with the same female.

Matings with ephippial females lasted from approximately 30 minutes to 1 hour. During that time only one egg was released into the ephippial case even though two are normally found in ephippia. Whether or not a second mating was required for release of the second egg is not known. None of the 20 matings that were observed were terminated before an egg was released into the ephippium.

Hybrid Genotypes

Table 5.1 shows the parent clones and genotypes for each hybrid. Examination of these data revealed no evidence of linkage between the three loci.

Unfortunately, only ephippia from crosses between English males and Arctic females were successfuly hatched even though viable ephippia from reciprocal crosses were obtained. Viability was assessedusing the tetrazolium

Table 5.1 Parent Clones and Genotypes of Arctic-English Hybrids

Hybrid	Parents		Genotype		
	English	Arctic	Pgi	Mdh	Lap
H1	SF	100	SF	MM	SF
H2	SF	100	SF	MF	SF
H3	SF	108	SF	MF	SF
H4	SF	108	FF	MF	SF
H5	SF	108	SF	MM	SF
H6	MF	108	SF	MF	SN
H7	MF	108	FF	MF	SM
H9	MF	108	FF	MM	SM
H11	MF	108	SF	MM	SM
H12	MF	108	FF	MM	SM
H13	MF	100	SF	MF	SN
H14	MF	100	FF	MM	SN
H15	MF	100	FF	MM	SN
H17	MF	100	FF	MM	SM
H18	SF	100	FF	MF	SF
H19	SF	100	FF	MF	SF
H20	SF	73	FF	MF	SF
H22	SF	73	FF	MF	SF
H23	MF	100	FF	MM	SN
H24	MF	100	SF	MM	SN
H25	MF	100	FF	MM	SM
H26	SF	108	FF	MM	SF

Table 5.1 Continued

Hybrid	Parents		Genotype		
	English	Arctic	Pgi	Mdh	Lap
H27	SF	108	SF	MF	SF
H28	SF	108	SF	MF	SF
H29	SF	108	SF	MM	SF
H30	SF	108	FF	MF	SF
H31	SF	108	SF	MM	SF
H32	SF	108	FF	MF	SF
H33	SF	73	SF	MF	SF
H34	SF	73	FF	MM	SF
H35	SF	73	SF	MM	SF
H36	SF	73	FF	MM	SF
H37	SF	73	SF	MF	SF
H38	SF	73	FF	MM	SF
H39	SF	73	FF	MF	SF
H40	SF	73	SF	MM	SF
H41	SF	73	FF	MM	SF
H43	SF	73	FF	MF	SF

test described in Chapter VI. Of 20 ephippial eggs from crosses between Arctic males and English females tested, 17 turned red indicating that they were viable.

Analysis of the banding patterns of PGM, GOT and ALK-2 in the hybrids confirmed earlier reports on the structure of these enzymes in Daphnia. PGM was thought to be a monomer from work on asexual populations of D. pulex (Chapter III). The hybrids had a two-banded PGM pattern which showed this assumption to be correct.

Young (1979a) and Hebert (1972) had found triple-banded variants of GOT and ALK-2 respectively. The hybrids also had three-banded patterns confirming that these enzymes are dimers.

All hybrid offspring of SF males had a double-banded Lap pattern (the slow Arctic and fast SF band) indicating that this enzyme is a monomer. Half of the offspring of MF males only had the slow Arctic band. The other half were double-banded but the fast SF band was replaced by a band of medium mobility. This indicated that the MF clone was heterozygous for an M allele and a Nul allele while the SF clone was homozygous for an F allele.

The number of bands could not be determined in the hybrid G6PDH pattern but it was evident that hybrid enzyme was being produced. The parent bands were wide enough apart so that three bands should have been distinguishable if the enzyme was a dimer. Most studies of G6PDH in other organisms have indicated that it is a dimer (Noltmann and

Kuby, 1963) however Yoshida (1966) found that G6PDH from human erythrocytes was a hexamer. When the molecule is dissociated it first breaks into two trimers which show only weak activity. The present study of D. magna suggests that G6PDH in Daphnia may also have more than two subunits. Attempts to increase the separation of the bands by using 10% gels and/or by increasing the running time were not successful.

It is interesting to note that despite the large genetic differences between the English and Arctic Daphnia, subunits of multimeric enzymes from the different strains were still able to combine and form an active hybrid enzyme. This is not suprising however, in light of the work done by MacIntyre and Dean (1978) on Acid phosphatase-1 in Drosophila. They extracted the enzyme from 11 different species and then dissociated it into its subunits. Several members of different species complexes were included. Dissociated subunits were mixed and allowed to re-associate in 41 interspecific combinations. In all cases a hybrid enzyme was formed. They found that amino acid substitutions had occurred in the contact sites and these differences often caused changes in the affinity of interspecific subunits for one another. In some cases the affinity was even increased. Therefore, genetic divergence had not progressed to the point where dimerization failed to occur even between groups of species that had been separated for extremely long periods of time. This shows that extensive diff-

erentiation can occur without causing the loss of binding between interspecific enzyme subunits.

The Arctic EST-1 pattern consisted of five bands of variable intensity. The mode of inheritance of this enzyme in Arctic clones could not be determined. It was already known from the English populations that homozygotes had two bands and heterozygotes had four indicating a monomeric structure for this enzyme. But, when the hybrids were examined, it appeared as though the English pattern was superimposed on the multibanded Arctic pattern resulting in an uninterpretable blur. Further analysis must be done before the relationship between the English and Arctic Est-1 patterns can be determined. The differences reflect a more complex situation than simple gene substitution such as was the case in the other enzymes studied. Extensive qualitative changes have occurred at this locus between the two strains.

Competition Experiments

All the hybrids that hatched survived to maturity and were fertile. Six of the hybrids; H1, H7, H11, H18, H26 and H28, from three different types of matings, were chosen for the competition experiments. The genotypes of these hybrids were shown in Table 5.1.

The results of these experiments are shown in Table 5.2. Results are expressed as the proportion (column four) of the clone in column one that remained in the jar when it

was competed with the competitor in column two. In this table clones are always parents and competitors are always hybrids. The letter in column five is based on the average of the two replicate jars. A 'W' indicates that 50% or more of the animals remaining were parent clones. An 'L' indicates that 50% or more of the animals remaining were hybrid competitors. Capitals indicate competitive exclusion, lower case letters indicate coexistance between the two strains in the jar.

In the first analysis each parent clone was compared to each of its hybrid offspring using a two-way analysis of variance with competitor and temperature as main effects. A Duncan's multiple range test was also performed to rank each competitor with respect to its ability to compete with the parent clone. Table 5.3 shows the results of these analyses. Graphs of these results are shown in Figure 5.1.

SF versus Hybrids

When clone SF was compared to its offspring H1, H18, H26 and H28, a highly significant competitor effect was found ($F=16.57$, $p=0.0009$). The Duncan's multiple range test shows that SF was most successful when competed against H1 and least successful against H26 with H18 and H28 falling in the middle. There was also a significant temperature effect ($F=8.38$, $p=0.02$) which can be explained by referring to Table 5.2. It seems that SF tended to "win" at 10°C but "lose" at room temperature (r.t.).

108 versus Hybrids

Only a significant competitor effect was found in competition experiments between 108 and its offspring ($F=5.61$, $p=0.0229$). Clone 108 tended to "win" or coexist with H11, H7 and H28 but was excluded by H26. Results for all four competitors were fairly consistent between the two temperatures accounting for the lack of a significant temperature effect.

100 versus Hybrids

The results of competition between 100 and its offspring show a strong temperature effect ($F=69.36$, $p=0.0011$). Clone 100 tended to do very poorly in competition at 10°C but was the "winner" at r.t.. This was true for both competitors H1 and H18.

MF versus Hybrids

Clone MF tended to do well at 10°C but did very poorly at r.t. in competiton with H11 and H7 ($F=14.12$, $p=0.0918$). Results for the two competitors were not significantly different from one another.

From these results it is apparent that the parent clones differ in their abilities to compete with their hybrid offspring and that in some cases temperature may have a decisive effect on the outcome.

I was also interested in looking at the outcome of

Table 5.2 Results of Competition Experiments With Respect to Clones
(see page 139 for explanation of column 6)

Clone	Competitor	Temperature	Proportion (by Clone)	Mean	Outcome (by Clone)
SF	H1	10°C	1.00 1.00	1.00	W
		room	0.906 1.00	0.953	W
	H18	10	0.00 0.75	0.375	1
		room	0.00 0.45	0.225	1
	H26	10	0.121 0.00	0.061	L
		room	0.00 0.00	0.00	L
	H28	10	0.841 1.00	0.921	W
		room	0.00 0.00	0.00	L

Table 5.2 Continued

Clone	Competitor	Temperature	Proportion (by Clone)	Mean	Outcome (by Clone)
MF	H7	10°C	0.979 1.00	0.99	W
		room	0.00 0.00	0.00	L
	H11	10	0.861 0.00	0.431	1
		room	0.00 0.00	0.00	L
100	H1	10	0.00 0.00	0.00	L
		room	1.00 1.00	1.00	W
	H18	10	0.25 0.125	0.188	L
		room	0.813 1.00	0.907	W

Table 5.2 Continued

Clone	Competitor	Temperature	Proportion (by Clone)	Mean	Outcome (by Clone)
108	H26	10°C	0.00 0.00	0.00	L
		room	0.522 0.219	0.371	1
	H28	10	1.00 0.75	0.875	w
		room	0.529 0.375	0.452	1
	H7	10	0.958 1.00	0.979	W
		room	0.65 0.60	0.625	w
	H11	10	1.00 0.629	0.815	w
		room	0.654 0.974	0.814	w

Figure 5.1 Results of Competition Between Parent Clones and Hybrid Offspring

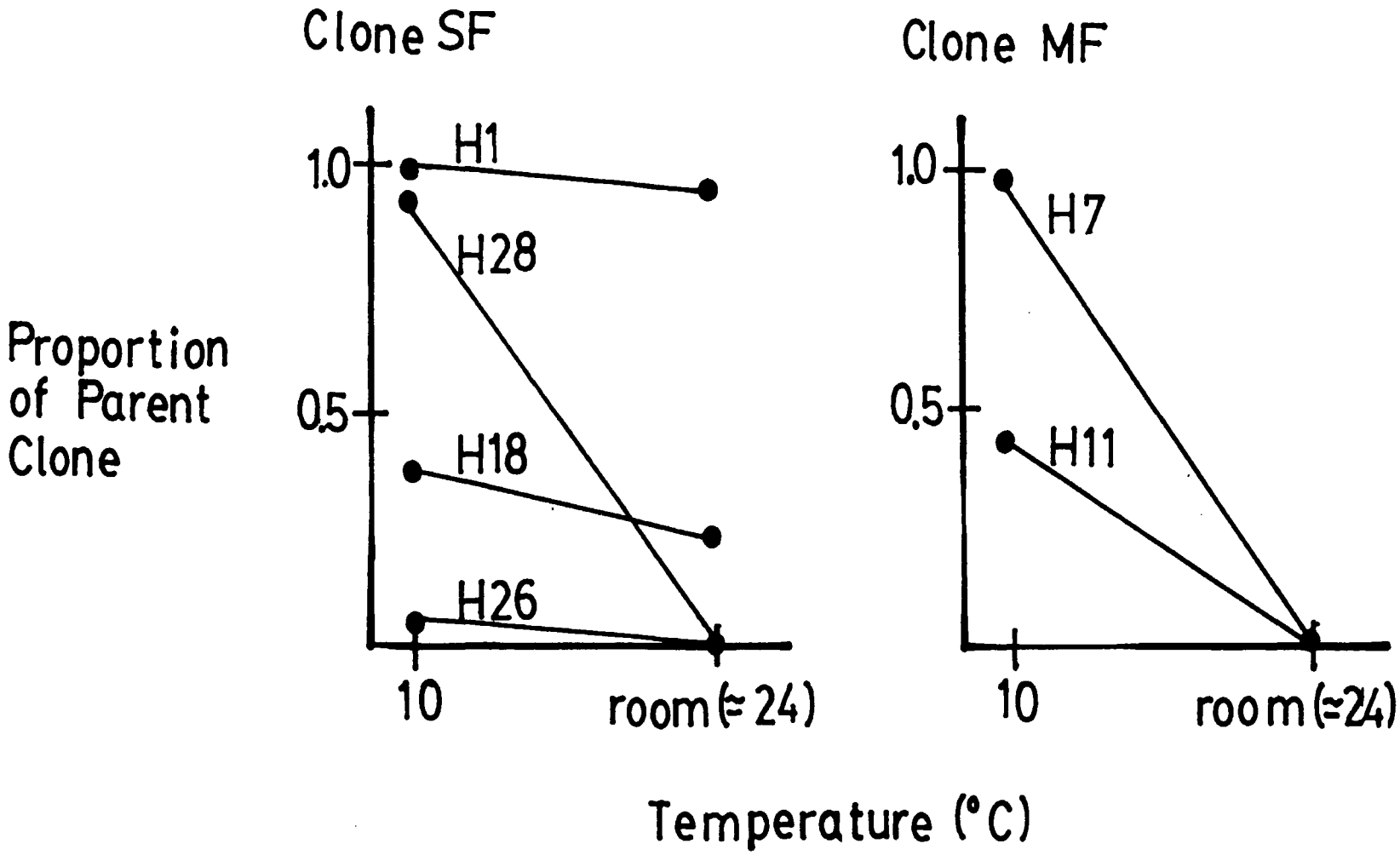


Figure 5.1 Continued

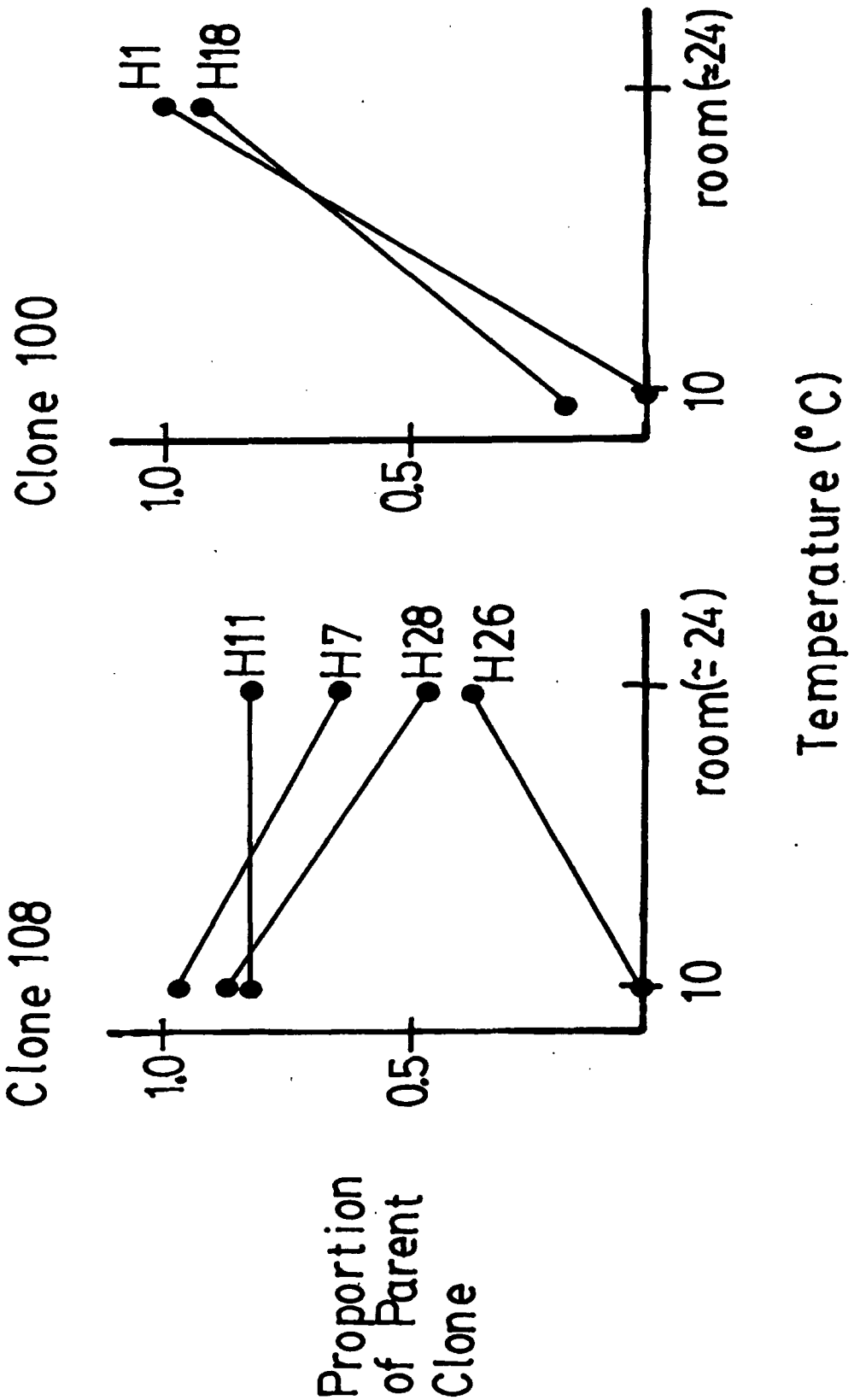


Table 5.3 Two-Way ANOVA for Clones With Competitor and Temperature as Main Effects.

Duncan's multiple range test shown below each ANOVA. Competitors are ranked from worst to best (alpha=.05).

a. Clone SF N=16

Source	D.F.	Mean Square	F Value	pr > F
Competitor	3	1.522	16.57	0.0009
Temperature	1	0.77	8.38	0.02
Interaction	3	0.323	3.52	0.0688
Error		0.092		

D.F.=8

Grouping	Competitor
A	H1
B	H28
C B	H18
C	H26

b. Clone MF N=8

Source	D.F.	Mean Square	F Value	pr > F
Competitor	1	0.451	3.23	0.1469
Temperature	1	1.974	14.12	0.0918
Interaction	1	0.451	3.23	0.1469
Error		0.14		

D.F.=4

Grouping	Competitor
A	H7
A	H11

Table 5.3 Continued

c. Clone 100 N=8

Source	D.F.	Mean Square	F Value	pr > F
Competitor	1	0.007	0.17	0.7206
Temperature	1	3.49	69.36	0.0011
Interaction	1	0.125	2.48	0.1903
Error		0.05		

D.F.=4	Grouping	Competitor
	A	H1
	A	H18

d. Clone 108 N=16

Source	D.F.	Mean Square	F Value	pr > F
Competitor	3	0.679	5.61	0.0229
Temperature	1	0.361	2.98	0.1226
Interaction	3	0.302	2.49	0.1345
Error		0.121		

D.F.=8	Grouping	Competitor
	A	H11
	A	H7
	A	H28
	B	H26

competition for each hybrid with respect to its two parent clones. A two-way analysis of variance with clone and temperature as the main effects was performed separately for each of the six hybrids. The results are shown in Table 5.5. Table 5.2 has been re-arranged to give Table 5.4 so that results are grouped according to competitor rather than clone. Figure 5.2 shows the results graphically.

H1

The results of the ANOVA for H1 and its parent clones, 100 and SF, show that all three effects; clone, temperature and interaction were significant. H1 did very poorly against SF at both temperatures. It was also excluded by clone 100 at r.t. but managed to completely exclude clone 100 at 10°C. The interaction term reflects the fact that H1's success cannot be predicted at 10°C unless one also knows with which clone it is competing.

H18

The other offspring of an SF by 100 mating showed nearly opposite results. In contrast to H1, H18 tended to do fairly well against both parent clones except when competed with 100 at r.t.. In this case it was nearly excluded. Due to the large variance in values, neither the clone or temperature effects were significant however the interaction effect was nearly significant at the 0.05 level ($F=4.75, p=0.09$).

H26

None of the main effects were significant because H26 seemed to outcompete both parent clones (SF and 108) at both temperatures. Clone 108 did manage to coexist with H26 at low frequency at r.t. but overall H26 was a very good competitor.

H28

There was a significant temperature effect ($F=18.63$, $p=0.0125$) in the the experiments with H28 against SF and 108. This was due to the fact that H28 tended to be excluded by both parents at 10°C while it excluded SF and coexisted in nearly equal frequency with 108 at r.t..

H11

None of the main effects were significant in the case of H11 versus MF and 108 however this was, in part, due to the variance in replicates. The competitor effect was nearly significant at the the 0.05 level ($F=4.72$, $p=0.0956$) which would have been expected from looking at the results in Table 5.4. In general H11 did fairly poorly against clone 108 but excluded clone MF at r.t.. Note, however, the large disparity in outcomes against MF at 10°C. In one replicate H11 excluded MF but in the other jar MF nearly excluded H11. Overall, H11 appeared to show greater competitive ability against MF than against 108.

Table 5.4 Results of Competition Experiments With Respect to Hybrid Competitors

Competitor	Clone	Temperature	Mean Proportion (by Competitor)	Outcome (by Competitor)
H1	SF	10°C	0.00	L
		room	0.047	L
	100	10	1.00	W
		room	0.00	L
H18	SF	10	0.625	W
		room	0.775	W
	100	10	0.812	W
		room	0.093	L
H7	MF	10	0.01	L
		room	1.00	W
	108	10	0.021	L
		room	0.375	1
H11	MF	10	0.569	W
		room	1.00	W
	108	10	0.185	1
		room	0.186	1

Table 5.4 Continued

Competitor	Clone	Temperature	Mean Proportion (by Competitor)	Outcome (by Competitor)
H26	SF	10	0.939	W
		room	1.00	W
	108	10	1.00	W
		room	0.629	w
H28	SF	10	0.079	L
		room	1.00	W
	108	10	0.125	l
		room	0.548	w

Figure 5.2 Results of Competition Between Hybrid Clones and Parent Clones

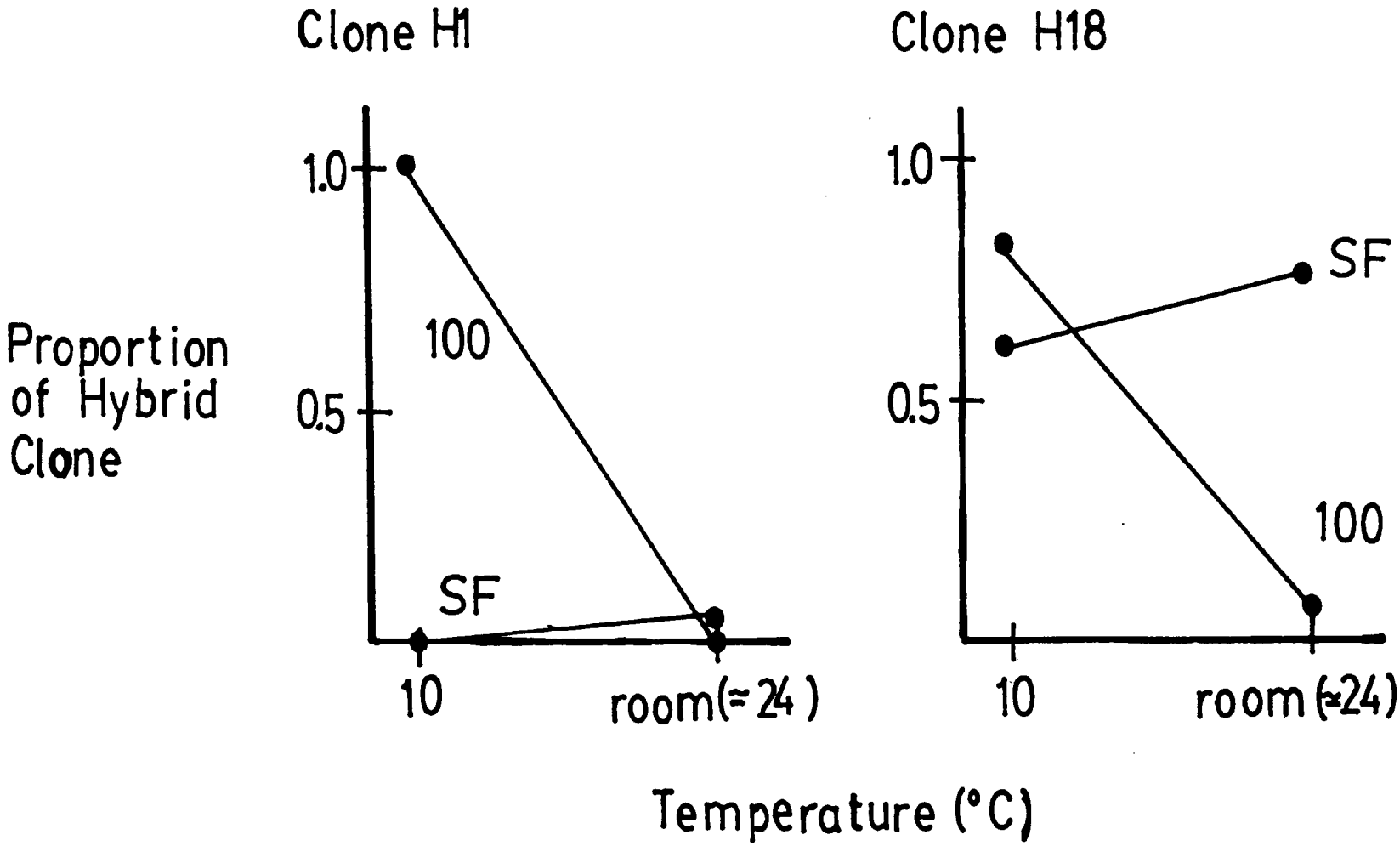


Figure 5.2 Continued

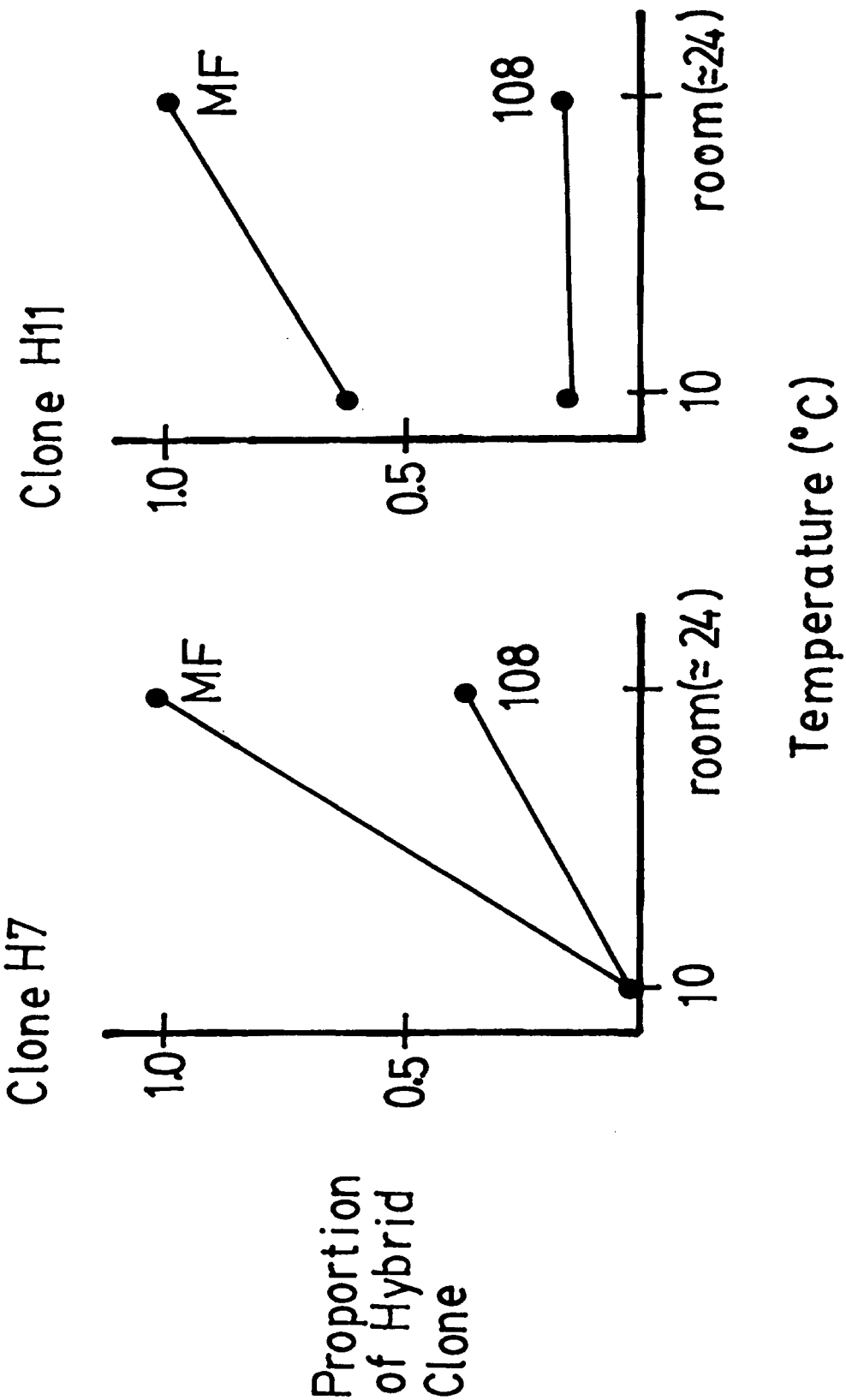


Figure 5.2 Continued

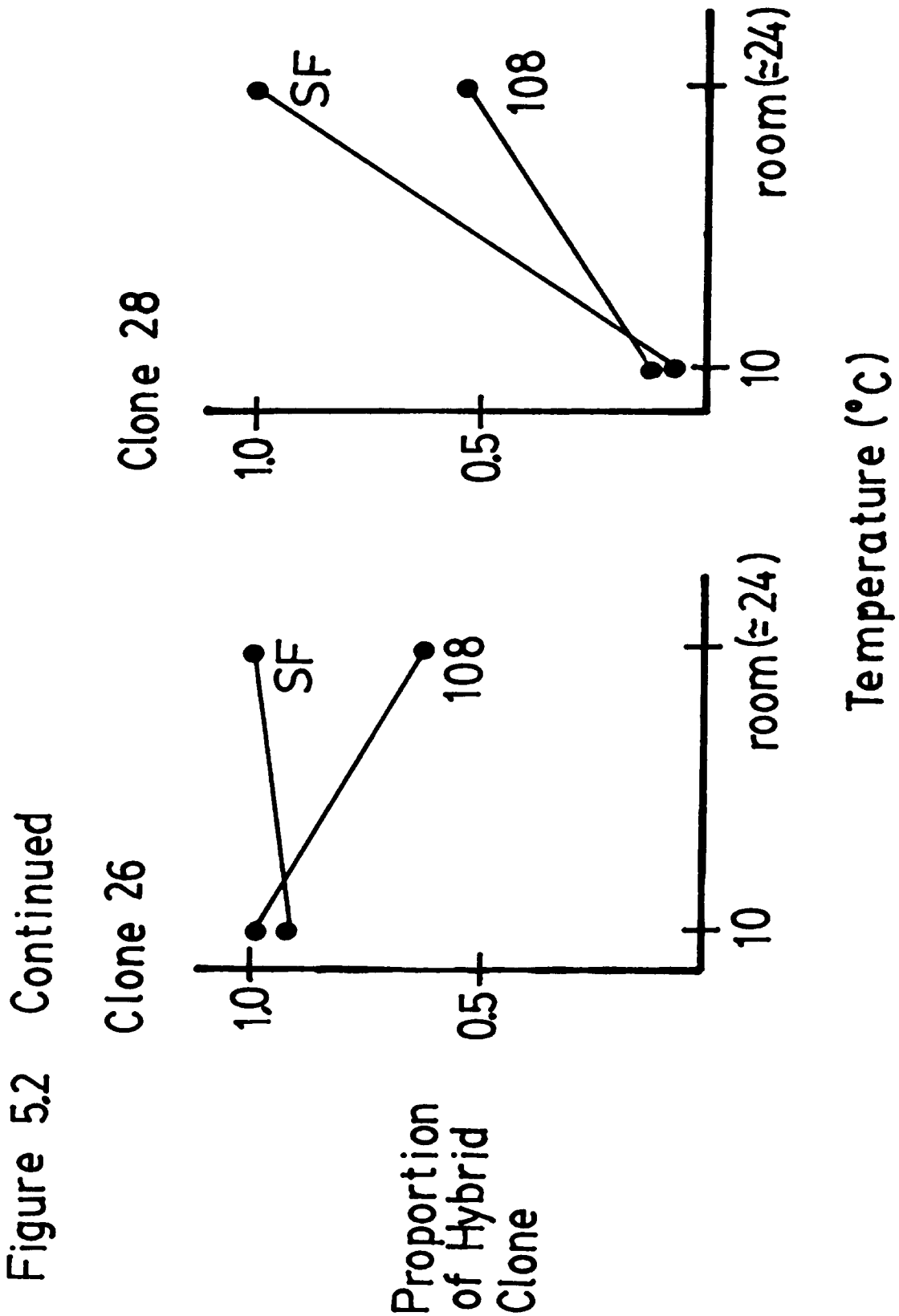


Table 5.5 Two-Way ANOVA for Competitors With Clone and Temperature as Main Effects

Duncan's multiple range test shown below each ANOVA. Clones are ranked from best to worst. (alpha=0.05)

a. Competitor H1 N=8

Source	D.F.	Mean Square	F Value	pr > F
Clone	1	0.914	38.29	0.0035
Temperature	1	0.914	38.29	0.0035
Interaction	1	1.601	67.04	0.0012
Error		0.024		

D.F.=4

Grouping	Clone
A	SF
B	100

b. Competitor H18 N=8

Source	D.F.	Mean Square	F Value	pr > F
Clone	1	0.313	1.87	0.2431
Temperature	1	0.388	2.31	0.2028
Interaction	1	0.796	4.75	0.0947
Error		0.167		

D.F.=4

Grouping	Clone
A	100
A	SF

Table 5.5 Continued

c. Competitor H7 N=8

Source	D.F.	Mean Square	F Value	pr > F
Clone	1	0.200	12.24	0.0249
Temperature	1	0.46	150.38	0.0003
Interaction	1	0.258	15.77	0.0165
Error		0.016		

D.F.=4	Grouping	Clone
	A	108
	B	MF

d. Competitor H11 N=8

Source	D.F.	Mean Square	F Value	pr > F
Clone	1	0.053	3.43	0.1375
Temperature	1	0.053	3.43	0.1375
Interaction	1	0.099	6.48	0.0636
Error		0.015		

D.F.=4	Grouping	Clone
	A	108
	A	F

Table 5.5 Continued

e. Competitor H28 N=8

Source	D.F.	Mean Square	F Value	pr > F
Clone	1	0.078	0.71	0.4465
Temperature	1	2.046	18.63	0.0125
Interaction	1	0.149	1.36	0.3085
Error		0.11		

D.F.=4	Grouping	Clone
	A	108
	A	SF

f. Competitor H26 N=8

Source	D.F.	Mean Square	F Value	pr > F
Clone	1	0.53	3.43	0.1375
Temperature	1	0.53	3.43	0.1375
Interaction	1	0.099	6.48	0.0636
Error		0.015		

D.F.=4	Grouping	Clone
	A	108
	A	SF

H7

All three main effects were significant for H7 versus MF and 108. The temperature effect was especially apparent. H7 tended to be excluded by both parent clones at 10°C but either excluded or coexisted with its parent clones at r.t.. The outcomes at r.t. also account for the significant difference between clones and for the significant interaction effect.

Table 5.6 shows the result of a Duncan's multiple range test if all six hybrids are ranked with respect to 1) English parents (SF, MF) 2) Arctic parents (100, 108) and 3) overall. The order of hybrids from worst to best is quite different when Arctic and English clones are considered separately. There was a significant temperature effect only when the hybrids were compared to the English clones which showed higher competitive ability at 10°C than at r.t.. There was a significant interaction effect in all three ANOVA's which reflects the heterogeneity in hybrid competitive ability.

F_2 offspring were hatched in early 1980. One was produced by the H1 hybrid clone and the other by the H2 hybrid clone. Both of these offspring were viable and produced many large parthenogenetic broods. No analysis of these F_2 has been done as of the present.

Table 5.6 Two-Way ANOVA for Pooled Results and Corresponding Duncan's Multiple Range Test.

Competitors are ranked from worst to best (alpha=0.05)

1. English Parents N=24

Source	D.F.	Mean Square	F Value	pr > F
Competitor	5	1.018	9.44	0.0008
Temperature	1	2.333	21.64	0.0006
Interaction	5	0.366	3.39	0.0384
Error		0.108		

D.F.=12

Grouping	Competitor
A	H1
B	H7
B	H28
C B	H18
C B	H11
C	H26

Grouping	Competitor
A	10
B	20

Table 5.6 Continued

2. Arctic Parents N=24

Source	D.F.	Mean Square	F Value	pr > F
Competitor	5	0.41	4.21	0.0193
Temperature	1	0.346	3.55	0.0841
Interaction	5	0.907	9.30	0.0008
Error		0.098		

D.F.=12

Grouping	Competitor
A	H11
A	H7
A	H28
A	H1
A	H18
B	H26

Grouping	Temperature
A	20
A	10

Table 5.6 Continued

3. Overall N=48

Source	D.F.	Mean Square	F Value	pr > F
Competitor	5	0.948	4.09	0.0049
Temperature	1	0.441	1.9	0.1763
Interaction	5	1.122	4.84	0.0017
Error		0.232		

D.F.=36

Grouping	Competitor
A	H1
A B	H7
A B	H28
A B	H11
C B	H18
C	H26

Grouping	Temperature
A	10
A	20

DISCUSSION

The willingness with which D. magna males from both England and the Arctic mated with females from the other group shows that little or no sexual or mechanical isolation between the groups has occurred. This is not suprising however, as matings between closely related species of Daphnids have been observed on several occassions. Agar (1920) successfully produced offspring from matings between D. pulex and D. obtusa in the laboratory. We have observed matings between D. magna and D. similis in the laboratory as well. Daphnia males are not very discriminating in their choice of mates so that reproductive isolation between two closely related species would more than likely evolve through ecological or post-zygotic mechanisms.

Although ephippia were obtained from all types of matings, only those from crosses between Arctic females and English males were successfully hatched. Unfortunately, D. magna ephippia of any sort were difficult to hatch in the laboratory. We even had difficulty hatching ephippia produced by matings between individuals within clones and within groups. Until a more reliable method for hatching ephippia is found it cannot be determined whether or not hybrid ephippia from English females and Arctic males are capable of hatching. A few ephippial eggs from such crosses were tested using the tetrazolium staining procedure

to check for viability (see Chapter VI). The eggs did turn red indicating that they are viable. All that remains is to find a way to hatch them.

General observation of all the hybrids that did hatch proved them to be viable when reared alone. Woodrich (1980, in prep) determined intrinsic rates of increase for the first five hybrids that hatched. Their 'r' values, although higher than the Arctic and English parents at three temperatures (10, 20 and 30°C) were only significantly higher than the Arctic parents at 10°C. We were able to hatch a single inbred ephippium produced by a clone from the Arctic 103 population and, in contrast to the hybrids, this female proved to be sterile. She did not produce a single parthenogenetic offspring during her life.

The fact that the hybrids were capable of reproducing asexually does not necessarily indicate that they were capable of sexual reproduction. Asexual reproduction in Daphnia involves only a single maturational division very similar to mitosis so that chromosome compatibility for synapsis etc. would not be necessary (Zaffagnini and Sabelli, 1972). In order for hybrids to persist in nature, especially in temporary ponds, they would also require the ability to reproduce sexually. The hatching of two F_2 ephippia, one from the H2 clone and one from the H1 clone showed that these two hybrids, at least, were fertile. The two F_2 clones have not been studied further so it is not known how their 'r' values compare with the English and Arc-

tic and F_1 hybrid clones. So far, no ephippia have been produced by the F_2 hybrids so it is not known if they are capable of sexual reproduction.

Although English and Arctic Daphnia have not diverged far enough to cause hybrid sterility, it is not known at this time whether "hybrid breakdown" in the F_2 and further generations will occur. However, the two strains have clearly not diverged even to the semispecies level with respect to reproductive isolation even though they show as much genetic differentiation as sibling species of Drosophila . At best the two strains of Daphnia could be considered races or subspecies, recognized on the basis of their distinct genotypes.

Mettler and Gregg (1969) have suggested that races often form in species that are distributed in disjunct populations and have adapted to dissimilar environments. When the races meet in nature however, there is a tendency for the differences to diminish as hybridization occurs. The real test of whether or not Arctic and English Daphnia have diverged to the species level would be to combine an English and Arctic population in nature. If they tended to remain separate, with two distinct gene pools, instead of combining to form a single new population, it would suggest that sufficient isolating mechanisms have evolved to consider the two strains as separate species.

The results of the competiton experiments between the

hybrids and their parents showed that the hybrid clones varied with respect to their ability to compete with the clones that produced them. Some hybrids, for example H26, seemed to be very successful in competition against both parent clones while the competitive ability of others changed depending on the temperature and the parent clone with which they were competing. Woodrich (1980, in prep) did similar experiments with H1 and H4 at three temperatures (10, 20 and 30°C) and found that H4 was an excellent competitor at all temperatures, against both parents while, as in the present study, the results with H1 varied with parent and temperature.

These results confirm the idea that increased heterozygosity alone does not automatically confer increased fitness on hybrid offspring (Wallace, 1955; Mayr, 1963; Zali and Allard, 1976). At the same time the fact that some hybrids were produced that did appear to be superior to both parents indicates that hybridization between the two strains is potentially advantageous.

Hebert (1972, 1974a,c), Hebert and Ward (1976) and Young (1979b) found that heterozygote excesses were common at several allozyme loci in natural populations of Daphnia magna in England. Hebert found heterozygote excesses at the Mdh locus in nine out of 15 populations and at the Est-1 locus in five out of 11 populations. He also found a highly significant heterozygote excess at the To locus in one population and a slight, nonsignificant excess in another.

In addition, one pond he found to be polymorphic for the Alk-2 locus also showed a very large heterozygote excess. Young has also seen heterozygote excesses at the Got locus as well as at the Mdh and Est-1 locus. They argued that these heterozygote excesses did not necessarily indicate that selection was acting on these loci in particular but that it may reflect how selection is acting on the genome as a whole.

Several workers (Ohta and Kimura, 1971; Frydenberg, 1963) have shown that if there is selection for heterozygotes at one locus there will be apparent selection for loci in disequilibrium with it. This phenomenon has been termed "associative overdominance". Prolonged clonal reproduction, such as that seen in permanent populations of D. magna does often lead to severe linkage disequilibrium. Furthermore, Strobeck (1979) has shown that if there is selection for heterozygotes at a locus there will be apparent selection not only at closely linked loci but for other loci throughout the genome.

Therefore, heterozygote excesses at loci which do not appear to be heterotic themselves, may reflect increased heterozygosity throughout the genome as a whole. Hebert et al. (1980, in prep) have suggested that hybridization between inbred lines may be a source of this heterozygosity and that heterosis may be responsible for maintaining it. Daphnia populations have a fairly high level of inbreeding in that mating is more likely to occur between members of

the same clone than between members of different clones due to variance in clone sizes and differences in the timing of sexual reproduction (Young, 1979b). The result of this is that each clone tends to accumulate its own array of deleterious alleles which become homozygous with prolonged inbreeding. If by chance two genetically different clones were to outbreed, the offspring would be considerably more heterozygous than their parents. The increased fitness of heterozygotes (as indicated by heterozygote excesses) may be a result of overdominance or it may be the result of "synthetic heterosis" such as that described by Young (1979b). If there is selection against a recessive allele heterozygotes at associated loci will appear to be intermediate. Several such loci in repulsion equilibrium (such as might occur when two inbred lines are crossed) would cause an overall heterotic effect.

The hybridization between lines as divergent as the English and Arctic clones (recall genetic similarity between them was only about 0.6, Chapter IV) is an extreme case but, as mentioned earlier, the fact that some of the hybrids did seem to be superior to both parents does suggest that a strategy of outcrossing to genetically distinct lines may be advantageous.

It still remains to be seen whether or not the English-Arctic hybrids will maintain their increased viability. Wallace (1955) crossed individual of Drosophila melanogaster from five populations from New York, California,

Virginia, Israel and Chile. He showed that while interpopulational F_1 hybrids had increased viability (as measured by larva to adult survival under low nutritional stress), it began to diminish in subsequent generations. The decrease in viability was due to recombination between haploid genomes from the different populations. Flies whose haploid sets were derived from two different populations were less viable than flies with unassorted chromosome sets. Wallace suggested that the recombination was breaking down the unique co-adapted gene complexes that had evolved in each population. It is possible that the same phenomenon could occur in the D. magna hybrids as well. Examination of the F_2 offspring should help provide some insight into the problem.

SUMMARY

In order to document that the English and Arctic strains of D. magna were still capable of interbreeding, hybridization experiments were carried out. Ephippia containing eggs were obtained from all crosses between Arctic and English individuals. Only ephippia produced by Arctic females and English males were successfully hatched, however. In order to determine if eggs from reciprocal crosses were viable, tetrazolium staining was used. Eighty-four percent of the eggs tested stained indicating that they were viable.

All hybrids that hatched reached maturity and were fertile. Due to the large genetic differences between the two strains, hybrid offspring were considerably more heterozygous than either parent. Hebert's (1972) results with English D. magna suggested that fitness increased with increased heterozygosity. In order to compare the fitness of these interstrain hybrids with the parent strains, competition experiments between six hybrids and each of their parent clones, at two temperatures, were carried out. The results of the experiments were extremely varied with some hybrids being superior to both parents at both temperatures and others being inferior in all situations. Other hybrids possessed variable competitive ability depending on the temperature and the parent clone with which

it was competing.

These results indicated that while increased levels of heterozygosity do not automatically confer increased fitness, outcrossing to genetically diverse clones is potentially advantageous. It allows the construction of novel genotypes within a population, some of which may have higher fitness than the clones that produce them.

CHAPTER VI

INDUCTION OF SEXUAL REPRODUCTION IN *DAPHNIA MAGNA*

CHAPTER VI

INTRODUCTION

Much work in populations genetics requires the use of breeding studies. Such studies are essential to establish the inheritance of electrophoretic variants or to look for evidence of reproductive isolation between different populations. Some of the experiments with Daphnia magna described in Chapters V and VII required the use of large numbers of sexual females and males. Normally, D. magna reproduces parthenogenetically with the offspring being female and genetically identical to their mothers. Only under certain environmental conditions do females produce haploid sexual eggs and males. As a result, it was necessary to find a method for inducing large numbers of females to produce ephippia and males. Several people have studied the effects of environmental stimuli on sexual reproduction in Daphnia. Stross and Hill (1968) and Stross (1969) found that the production of ephippia in D. pulex required both inductively short photoperiods (L:D=12:12) and high culture densities. In short days the number of ephippia produced was directly proportional to the density of the culture.

Stimpfl (1971) found that four variables; photoperiod, food concentration, culture density and temperature inter-

acted in a complex way to cause ephippia production in D. pulex. Short-day photoperiods (L:D=9:15 and 12:12) coupled with low temperature (10^{o}C) and high culture density (up to eight animals per 24 mls) appeared to be most successful.

From this work it was apparent that high culture density during short photoperiods was the most successful combination for the induction of sex in Daphnia pulex. Based on this information several experimental regimes were tried with both English and Arctic clones of D. magna in an effort to produce large numbers of ephippial females and males. This chapter describes the results of these experiments.

MATERIALS AND METHODS

Clones SF, 108 and 203 were used in all the experiments. Stock cultures containing 10 to 15 adult females were maintained in 1½ liter jars using the techniques described in Chapter II. Offspring were removed and sexed every Monday, Wednesday and Friday. Initially stock cultures were kept in six hour days at 20°C in an environmental chamber but the Arctic clones produced males in these short days. As a result the stock cultures were moved to 24 hour days at room temperature (approximately 24°C) so that only females would be produced.

To induce ephippia production, one to two day old females were isolated from the stock cultures and raised in one liter of artificial pond water. Each experiment involved 50, 75 or 100 females. Initially all experiments were run at 19°C in six hour days but success was limited in all clones. As a result the daylength in the chamber was decreased to four hours which proved to be successful for Arctic clones but not for English. It was discovered that English females occasionally produced ephippia in stock cultures kept in continuous light so the experiments with English females were run in 24 hour days to try and increase ephippia production.

The cultures were fed with 150 mls of the Scenedesmus/liver mixture three times per week. Other work by Woodrich

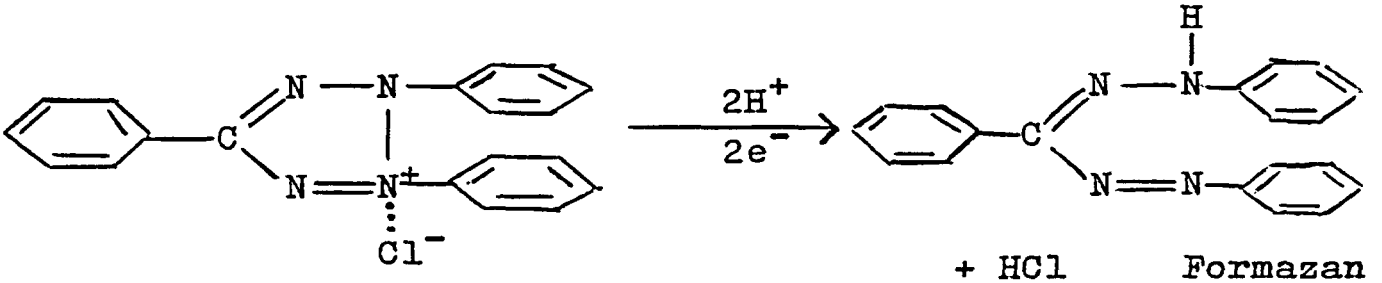
(1980) had shown that this was more food than necessary to allow healthy development of the juveniles. It was intended that food not be limited during maturation. When the females were mature, and carrying broods of parthenogenetic eggs, the culture volume was decreased to 500 mls and the food decreased to 80 mls in order to cause a rapid deterioration of the environment. Cultures were examined every Monday, Wednesday and Friday for the presence of ephippial females. All offspring were removed at this time. The maximum proportion of ephippial females observed during the experiment and the percent mortality at the time of maximum ephippial production was calculated for each experiment.

During these experiments it was noticed that some ephippia produced in the absence of males contained eggs; cyclic parthenogens are usually known to shed empty ephippia if fertilization does not occur (Agar, 1920). As a result all the ephippia produced during the course of these experiments were collected and opened to check for the presence of eggs. The percent containing eggs was recorded.

Hatching the ephippia was attempted by air drying them for one to two days and then placing them in 100 ml plastic beakers filled with artificial pond water. The beakers were kept at $0^{o}C$ for three weeks. Upon removal the ephippia were put into either a $10^{o}C$ or a $5^{o}C$ chamber with continuous lighting. After the ice had melted eggs were removed

from some of the ephippia and returned to the water while the remainder of them were left undisturbed. This method is similar to one used by Davison (1969) for D. pulex. No hatching was observed during the experiments.

Due to the difficulty in hatching ephippia it was decided to test for viability using tetrazolium staining. This test involves placing embryo tissue in a colorless solution of tetrazolium salt. If the tissue is alive hydrogen atoms will be released by the dehydrogenase enzymes involved in the respiration process. These hydrogen atoms will react with the tetrazolium salt to produce an insoluble red pigment called formazan. The staining reaction is as follows;



Triphenyl tetrazolium Chloride Formazan

The procedure described below is a modification of a test described by Moore (1973). The test was performed by placing eggs that had been removed from their ephippial cases onto a piece of #1 Whatman filter paper. The membrane surrounding the eggs was broken to allow penetration of the test solution. The filter paper was then moistened with a two percent solution of Triphenyl tetrazolium chloride in distilled water and allowed to incubate overnight at

20°C. If the egg turned red it was considered to be viable.

This test is most often used for testing the quality of seeds. Moore suggested that seeds be soaked in water before testing in order to activate their metabolic processes. The ephippia used in these tests were usually removed directly from Daphnia cultures and were thus already hydrated. If dehydrated ephippia were used, they were soaked for at least two days before being tested. Ephippia from several sources were tested using this method. Results were expressed as the percentage of viable eggs.

RESULTS

Ephippia Induction

The results of the ephippia induction experiments are shown in Table 6.1. All experiments with clones 203 and 108 were carried out in four hour days at 19°C. Preliminary experiments were done in this photoperiod with the SF clone but very few experiments yielded any ephippial females. In experiments where ephippial females were produced, Max_o (see below) was approximately five to 20%. As a result, most experiments with clone SF were carried out in 24 hour days. The results of these experiments are included in Table 6.1.

Two measures of ephippia production were recorded for each experiment. The maximum proportion of ephippial females with respect to original numbers (Max_o) is the highest number of ephippial females observed divided by the number of females used to start the experiment. The maximum proportion with respect to numbers present (Max_p) is the highest number of ephippial females observed divided by the number of females alive on that day. Percent mortality was calculated as the number females alive on the day of maximum ephippia production divided by the original number of females. The number of days required to reach maximum ephippia production was also recorded. These analyses are

Table 6.1 Results of Ephippia Induction Experiments.

Clone	Initial Number of Females	Number of Females at Maximum Ephippia Production	Number Ephippial	Number of Days
SF	50	45	18	14
	50	47	12	14
	50	48	9	14
	50	47	14	10
	50	49	21	14
	50	47	23	10
SF	80	71	18	14
	75	74	42	9
	80	78	23	12
	80	76	41	10
	75	62	21	14
	75	62	33	10
	75	63	14	9
	75	-	0	-
	75	-	0	-
SF	100	70	18	16
	100	91	24	17

Table 6.1 Continued

Clone	Initial Number of Females	Number of Females at Maximum Ephippia Production	Number of Ephippial Females	Number of Days
SF	100	82	29	15
	100	48	16	15
	90	84	48	10
	90	72	12	10
	100	-	0	-
	100	86	1	10
	100	90	2	10
				Mean=12.24
203	50	38	30	18
	100	76	24	16
	107	87	60	14
	80	71	38	15
	100	89	84	12
	100	96	80	12
	100	94	50	14
	100	95	75	16
	115	109	75	16

Table 6.1 Continued

Clone	Initial Number of Females	Number of Females at Maximum Ephippia Production	Number of Ephippial Females	Number of Days
203	65	60	51	13
	100	99	75	16
	75	72	9	14
	120	113	69	12
	98	85	48	14
	106	100	86	14
	100	96	72	13
				Mean=14.31
108	100	72	25	18
	100	91	47	14
	100	82	42	13
	105	102	91	15
	63	62	49	12
				Mean=14.4

shown in Table 6.2.

Max_o will underestimate the total number of females in an experiment that actually produced ephippia. Those females that went ephippial before or after the period of maximum ephippia production would not be included in the analysis. Fortunately, the growth of the animals in each jar was quite synchronous and only a few females produced their ephippia very early or very late during the course of the experiment.

Almost all of the experiments involving clone 203 were begun with 100 females. Upon reaching maturity most females produced at least one brood of parthenogenetic offspring (usually male) before becoming ephippial. The mean of Max_o was 64% with values ranging from 12 to 84%. The mean of Max_p was 76% with a range of 13 to 94%. The average mortality was 9% and the average number of days to maximum ephippia production was 14.3.

All but one of the experiments with clone 108 started with about 100 females. The last experiment only involved 63 females. The mean of Max_o was 56% with a range of 25 to 87% and the mean of Max_p was 61% (35 to 89%). The average mortality was 12% and the average number of days to reach maximum ephippia production was 14.4.

The experiments with the SF clone could be divided into three groups, those that were initiated with approximately 100, 75 or 50 females. A one-way analysis of var-

Table 6.2 Analysis of Ephippia Induction Experiments.

Clone	Max^*_o	Max^*_p	Mortality
SF	0.36	0.40	0.10
	0.24	0.26	0.06
50 Females	0.18	0.19	0.04
Initially	0.28	0.30	0.06
	0.42	0.43	0.02
	0.46	0.49	0.06
Mean	0.32	0.35	0.06
SF	0.23	0.25	0.11
	0.56	0.57	0.01
75 Females	0.29	0.28	0.03
Initially	0.51	0.54	0.05
	0.28	0.34	0.17
	0.44	0.53	0.17
	0.19	0.22	0.16
	0.00	0.00	-
	0.00	0.00	-
Mean	0.28	0.30	0.10
SF	0.18	0.26	0.30
	0.24	0.26	0.09
100 Females	0.29	0.35	0.12
Initially	0.16	0.33	0.52
	0.53	0.57	0.07
	0.13	0.17	0.09
	0.00	0.00	-
	0.01	0.01	0.14
	0.02	0.02	0.10
Mean	0.17	0.22	0.18
203	0.06	0.79	0.24
	0.24	0.32	0.24
	0.56	0.69	0.42
	0.48	0.54	0.11
	0.84	0.94	0.11
	0.80	0.83	0.04
	0.50	0.53	0.06
	0.75	0.79	0.05
	0.65	0.71	0.05
	0.78	0.85	0.08
	0.75	0.76	0.01
	0.12	0.13	0.04

Table 6.2 Continued

Clone	Max_o	Max_p	Mortality
203	0.58	0.61	0.06
	0.49	0.56	0.13
	0.81	0.86	0.06
	0.72	0.84	0.04
Mean	0.64	0.67	0.09
108	0.25	0.35	0.28
	0.47	0.52	0.09
	0.42	0.51	0.18
	0.87	0.89	0.03
	0.78	0.79	0.02
	0.56	0.61	0.12

* see page 177 for explanation of the terms Max_o and Max_p.

Table 6.3 One-way ANOVA for Results From Ephippia Induction Experiments With Clone SF.

a. Analysis of Max_o

Source	Mean Square	F Value	D.F.	pr F
Experiment	0.049	1.52	2	0.24
Error	0.032			

b. Analysis of Max_p

Source	Mean Square	F Value	D.F.	pr F
Experiment	0.035	0.89	2	0.43
Error	0.039			

c. Analysis of Mortality

Source	Mean Square	F Value	D.F.	pr F
Experiment	0.029	2.36	2	0.12
Error	0.012			

d. Analysis of Days to Maximum Ephippia

Source	Mean Square	F Value	D.F.	pr F
Experiment	6.372	0.96	2	0.401
Error	6.615			

iance was used to test whether there was a significant difference in the outcome of the three groups. All data expressed as proportions (Max_o, Max_p and Mortality) were arcsine transformed before analysis. The experiments with 50 females seemed to yield a higher proportion of ephippial females (mean Max_p=35%) with a lower mortality (6%) while the experiments with 100 females yielded the lowest number of ephippial females (mean Max_p= 22%) and the highest average mortality (18%). The results of the three groups, however, were not significantly different at the 5% level (Table 6.3) so the data from all experiments was pooled. The mean Max_o was 25% and the mean Max_p was 28%. The mean mortality was 12% and on average it took 12.24 days to reach maximum ephippia production.

The Release of Eggs Into Ephippia in the Absence of Males

Ephippia from these experiments and others where it was known for certain that the females were not exposed to males were collected and checked for the presence of eggs. D. Woodrich supplied some of these ephippia from her experiments and her help is gratefully acknowledged. The results of this analysis is shown in Table 6.4.

Twenty-one point five percent of clone 203's ephippia (203A) had eggs and of these 28% had two eggs, the remainder containing only one egg. Forty point four percent of the ephippia from clone 108 contained eggs with 36% of these having two. Only 19% of clone 73's ephippia had eggs

Table 6.4 Analysis of the Egg Content of Ephippia Produced in the Absence of Males

Clone	N	No Eggs	One Egg	Two Eggs	Percent of Total With Eggs
73	136	110	21	5	19.12
100	228	155	46	27	32.02
108	488	291	126	71	40.37
SF	412	411	1	0	0.24
203A	497	390	77	30	21.53
203B					
i. Females with early ephippia and eggs in the ovaries	201	146	37	18	27.36
ii. Females with eggs in their ephippia	159	66	81	12	58.49
iii. All 203B ephippia	680	443	192	45	34.85

and 19% of these contained two. Out of 412 SF ephippia collected only one was found to contain an egg.

These ephippia were obtained from different experiments run under different conditions. Another set of ephippia from clone 203 (203B) from more uniform conditions were checked for comparison. All of these ephippia came from the ephippia induction experiments described above. All the females were kept in experimental conditions until their ephippia were shed. Two categories of ephippial females were observed separately. Females found to have early ephippial cases and eggs in their ovaries were isolated from the others. Similarly females possessing an ephippium with an egg already in it were separated from the others. On three or four occasions I had observed females removing an egg from the ephippium with the abdominal claw. It was of interest to determine how often this occurred.

The results of these analyses were combined with those of other ephippia that were checked but were not observed during development. The results are shown in Table 6.4. Twenty-seven percent of the ephippia from females observed to have eggs in their ovaries during ephippial development contained eggs after they were shed, 34% of these had two eggs. Fifty-eight percent of the ephippia with eggs before release still contained eggs afterwards. Only 13% of these had two eggs. This shows that even though eggs are released into ephippia during their development, quite

a large proportion are lost before they are released. When all the 203 ephippia from these experiments were combined 34.9% were found to contain eggs and of these 19% had two.

I was not able to hatch any of these ephippia but the tetrazolium test indicated that some of the eggs might be viable. As a control, 29 eggs from English-Arctic matings (which were known to be viable due to the hatching of hybrids) were tested. Twenty-five (86.2%) of these turned red indicating viability. Ninety-six eggs from the 203 ephippia were tested and 29 (30%) turned red.

Male Production

During the course of these experiments it was discovered that the Arctic females were very sensitive to short photoperiods. Young females from clone 203 placed in four hour daylengths at 19°C produced virtually all male offspring upon reaching maturity. This occurred regardless of the density of the females. On the other hand, it was extremely difficult to induce English females to produce male offspring. Males were observed only in extremely dense cultures and even then the proportion of males was not very high. This was true for all three daylengths tested; four, six and 24 hours.

DISCUSSION

These experiments have shown that large differences exist in the reproductive response of English and Arctic D. magna to environmental stiumuli. Arctic Daphnia seem to be very sensitive to photoperiod, both in the production of ephippia and of males. Short daylengths tended to cause cultures to become sexual regardless of the state of the animals. When young females were raised in short days the usual pattern was to produce one, or sometimes two, parthenogenetic broods (almost always male) and then produce an ephippium. This occurred with as few as 65 healthy females in the jar. During development of the juvenile females, excess food was given to allow maximum growth. Once the animals were carrying parthenogenetic broods the food and culture medium was reduced to simulate rapid deterioration of the environment. But, in several cases, some of the Arctic females were beginning to produce ephippia even before the reduction occurred. It is not likely that this was caused by food shortage as the other females in the jar would be carrying large asexual broods.

A short day photoperiod was not essential to induce production of ephippia in Arctic females because they were occasionally observed in extremely crowded cultures grown in 24 hour days. These results are similar to those of Stross' (1969) experiments with D. middendorffiana from

Barrow, Alaska. He found that all broods were sexual at daylengths less than 20 hours. Furthermore, he found no evidence that crowding was necessary in permissive photoperiods. Even so, crowding was able to override the photoperiod response and cause the production of ephippia in females kept in continuous light.

Females from clone SF were much less sensitive to short daylengths than the Arctic clones and responded to a rapid decrease in the quality of their environment. In order for this to succeed, however, the females had to be very healthy prior to the change. Reducing the level of food and space for animals already exposed to suboptimal conditions had little or no effect. Furthermore, if the females did not respond soon after the change they would not respond at all. That is, if exposed to poor conditions for prolonged periods of time the females tended to either remain unreproductive or produce very small broods (usually less than five eggs) of parthenogenetic females.

Bunner and Halcrow (1977) found similar results in their work with D. magna. If females were maintained in inductive conditions for long periods of time they usually resumed asexual reproduction. Furthermore, it was not possible to induce a group of females to produce a second ephippium. If they increased population density or decreased food levels further, the only result was a dramatic increase in mortality.

The key to obtaining ephippial English females seems

to be to cause a sudden and drastic deterioration of the environment of extremely healthy, mature females. Timing and the condition of the females seemed to be the critical factors. Photoperiod had less effect although the experiments seemed to be more successful at 24 hours than at shorter daylengths. Why this should be so is not understood.

The differences between the two strains can be explained by considering their natural environments. The growing season in the Churchill area is very short, from the beginning of June to the end of August. When the environment begins to change, for example, cooler temperatures and shortening days, the female D. magna must produce ephippia before the populations die out. The English Daphnia used in these experiments were taken from a permanent pond which supports an adult population all year round. As a result sexual reproduction is not necessary on a regular basis. It is only advantageous to produce ephippia and males when conditions are rapidly deteriorating and the chance of survival is low due to the possible collapse of the population.

Another interesting feature of Arctic D. magna is the fact that they release unfertilized eggs into their ephippia. From 19 to 35% of the ephippia of various Arctic clones were found to contain one or two eggs. By contrast, only one of 412 (0.2%) ephippia produced by the English clone contained an egg. Of particular interest are the ephippia

from the experiments with clone 203. One hundred and fifty-nine females with eggs in their ephippia were isolated. When the ephippia were checked after being shed only 58% still contained eggs. Females had actually been observed extruding eggs from their ephippia. This data seems to indicate that nearly half of the eggs are discarded after release into the brood pouch. This would seem to be a great waste of energy and egg cytoplasm on the females' part. No reason for this behaviour can be suggested. If the eggs are not able to develop it would be more reasonable to resorb them than to release them into the ephippium and then destroy them. On the other hand, if they are viable it does not seem reasonable to destroy them at all. The tetrazolium staining test showed that only about 30% appear to be viable. Further work needs to be done on this test to determine whether or not it is reliable. It is not known if there are conditions that would cause an egg to stain even though it is not capable of full development.

If we assume these data to be close to the actual situation it remains to be seen how the females would avoid extruding viable eggs, if in fact they do.

The fact that Arctic D. magna release apparently viable sexual eggs without fertilization suggests that they may be capable of facultative sex. To date no Cladoceran has been shown to possess this mode of reproduction. All species are thought to either produce all ephippia sexually, as in the case of cyclic parthenogens, or asexually, as in the case

of obligate parthenogens. However, on the basis of the pattern of allozyme variation in a population of Simocephalus serrulatus studied by Smith and Fraser (1976), Hebert (in prep) suggested that they too may possess the ability to self-fertilize their sexual eggs. Extreme heterozygote deficiencies at several polymorphic loci indicated that severe non-random mating was occurring.

Little is known of the mechanism by which self-fertilization would occur in Daphnia, however some form of automixis is most probable. The ephippial eggs of Arctic females are known to undergo normal meiosis as shown by the production of English-Arctic hybrids. Zaffagnini and Sabelli (1972) have shown that in D. pulex the egg is released into the ephippium while it is still in Metaphase I. Release does not normally occur until after fertilization, however. Once in the ephippium the egg completes Meiosis I with extrusion of the first polar body which usually disappears in about ten minutes. Fusion of the pronuclei occurs sometime after Meiosis II. If this were also the case in D. magna self-fertilization would most likely be caused by the second polar body. In this situation the zygote would be homozygous at all loci (assuming no crossing over) even if the mother was heterozygous. But, if the first polar body did not degenerate immediately and itself underwent Meiosis II, there would be the possibility that one of its products may fertilize the eggs. In this case

the heterozygosity of the mother would be preserved.

The latter mechanism seems to be favored in obligate parthenogens reproducing by automixis. For example, in the Dipterans Drosophila mangabeirai (Carson, 1967) and Lonchoptera dubia (Stalker, 1956), and the moth, Solenobia triquetrella (Seiler, 1963), two non-sister nuclei fuse after meiosis thus preserving heterozygosity. The high homozygosity of the Arctic D. magna populations seems to favor the first alternative. In order to determine which mechanism is correct large numbers of asexual ephippia from females heterozygous for allozyme markers must be hatched. Many such ephippia were obtained from clone 203 which is heterozygous for Mdh however all attempts to hatch them were unsuccessful. Until this can be accomplished the question remains unresolved.

The evolution of facultative sex in Arctic Daphnia may be another adaptation to the short growing season. If fertilization does not occur it may be better to produce something, even if it is highly inbred, than nothing. Because of the lower temperatures and resulting increase in developmental time a female will probably be able to produce only one or two ephippia before the population dies out so she cannot risk her eggs being unfertilized. If mating did not occur within a certain period of time she would be able to fertilize them herself.

A number of other normally sexual organisms do in fact

possess the ability to reproduce asexually. Eight species of Drosophila are capable of producing adult progeny parthenogenetically. The species are D. parthenogenetica, D. polymorpha, D. affinis (Stalker, 1954); D. robusta (Carson, 1961); D. mercatorum (Carson, 1967); D. ananassae and D. pallidosa (Futch, 1973) and D. paulistorum (Ehrman, pers. comm. in Templeton, 1979). The mechanism in all cases is fusion of the first two cleavage nuclei which results in complete homozygosity of the entire genome. In D. mercatorum this occurs with a frequency of about 10^{-6} in nature (Templeton, 1979).

The eggs of many species of orthopteroid insects that are normally bisexual will begin to develop if fertilization does not occur. Two genera, Apotettix and Paratettix have been extensively studied by Nabours (1930) and Robertson (1931). Usually females are produced from these eggs. Analysis of these eggs has suggested that the first division occurs normally but the second one is prevented. Diploidy is restored in later stages of development when the two chromatids remaining after Meiosis I finally split into two separate, homologous chromosomes.

In certain environments, for example where finding a mate is difficult or where males are fragile and subject to disease (White, 1970), selection would favor an increase in the frequency of automixis until at some point it could become the sole mode of reproduction. Several people have

suggested that this is how parthenogenesis may have evolved in many automictic parthenogens. Maynard Smith (1978) has suggested, however, that in cases where automixis is of the type that causes increased homozygosity, sexual reproduction will not be lost entirely.

SUMMARY

During the course of this study it was often necessary to produce large numbers of D. magna ephippial females and males. Several experimental regimes designed to induce sexual reproduction were tried for both Arctic and English laboratory clones. Experiments involved setting up approximately 100 juvenile females in one liter of artificial medium and allowing them to reach reproductive maturity. At that time the volume of the culture was reduced by half as was the amount of food. Cultures were observed every other day to check for the presence of ephippial females.

The Arctic clones, 203 and 108, were extremely sensitive to short-day photoperiods. Almost all parthenogenetic offspring produced under conditions of four hour light and 19°C were male. In addition, the average maximum ephippia production recorded was 64% for clone 203 and 56% for clone 108. The photoperiod response could be overridden, even in continuous days, for if cultures became very crowded males and ephippia were produced.

The English clone, SF, was fairly insensitive to photoperiod. Cultures kept in four hour light produced few ephippia. Females from stock cultures kept in continuous days were occasionally observed to produce ephippia so the experiments were run under 24 hour light. The optimal stimulus for ephippia production in this clone was a sud-

den drastic decrease in the quality of the environment. In order for this to be successful however, the females had to be extremely healthy. Deterioration of the environment of females already under nutritional stress was uneffective. The average maximum ephippia production recorded was 26%. Males were produced only under extremely crowded conditions.

It was suggested that perhaps the induction of sexual reproduction in English individuals was particularly difficult because this clone originated from a permanent population where sex was not crucial on a regular basis. Arctic ponds, on the other hand, freeze every year so it essential that the populations produce ephippia when the environmental cues (such as decreasing photoperiod) signal the approach of winter.

During these experiments it was noticed that some Arctic ephippia produced in the absence of males contained eggs. Analysis revealed that a large percentage of the ephippia contained eggs; from 19% in clone 73 to 35% in clone 203. In contrast, only 0.2% of the ephippia produced by the English clone contained eggs.

Several methods were tried to induce the hatching of these ephippia, without success. As a result, tetrazolium staining was used to test for viability. Only about 30% of the unfertilized eggs produced by Arctic females appeared to be viable.

The discovery of asexual ephippial eggs in populations capable of sexual reproduction (production of English-Arc-

tic hybrids proved that normal meiosis was occurring in Arctic females) raises the possibility that these populations are capable of facultative sex. Automixis is a potential mechanism but ephippia from females heterozygous for enzyme markers must be hatched before the mechanism can be ellucidated. The production of automictic ephippial eggs by Arctic populations may be another adaptation to the short growing season.

CHAPTER VII

MATING ATTRACTION IN *DAPHNIA MAGNA*

CHAPTER VII

INTRODUCTION

Swarming behaviour has been noted in several Cladoceran species both in laboratory cultures and in nature. Several workers have suggested that these swarms may be related to sexual reproduction due to the fact that a high percentage of the swarming animals are often ephippial females. For example, Brandl and Fernando (1971) analysed aggregations of Ceriodaphnia affinis in a laboratory culture. After isolating a swarm of 486 females they found that 459 (95%) of them produced ephippia. Two hundred and fifty females from outside the swarm were also isolated and none of these produced ephippia. Santharam et al. (1977) found similar results when they studied swarming in Daphnia carinata. They collected animals from ponds near Madurai University in India and maintained the entire collection in enamel trays in the laboratory. After 48 hours a swarm developed in the center of the tray. After isolating the animals from this swarm they found that 97% (1204/1236) produced ephippia within 3 days. Animals collected from near the edge of the trays did not produce ephippia.

Ratzlaff (1974) analysed several swarms of Moina affinis from flood plain pools in Dauphin County, Penn-

sylvania. In one case he found that the swarm included 13.3% ephhippial females and 9.4% males. Outside the swarm males comprised 5.1% of the population but no ephippial females were found. Recently Young (1978) described swarming behaviour in a population of Daphnia magna in a pond near Cambridge, England. He noted that swarms were only seen during periods of sexual reproduction and contained large numbers of "imminently sexual" females and small numbers of males. Males were not found outside the swarms. He hypothesized that "females at a critical stage in sexual egg production are attracted towards males and that at least some males are attracted, probably by these females". Although Young does not describe the cause of this attraction, sex phermones seem to be the only explanation. The following experiments were designed to examine the possibility that either male or female D. magna attract one another through the production of sexual phermones.

MATERIALS AND METHODS

The experiments were run in aquaria containing three liters of artificial pond water and 300 mls of the Scenedesmus/liver mixture. The alga were well mixed into the water to insure that they were evenly distributed. The sides of the aquaria were covered with black paper and the bottom with white paper so that animals could be easily seen. The aquaria were centered under a fluorescent light to provide diffuse lighting that was as uniform as possible. Light intensity at the water's surface was 64 lux.

Males and ephippial females from Arctic clone 203 were used in these experiments. Males could be readily obtained from stock cultures kept in short day lengths. The production of ephippial females was described in detail in Chapter VI. Only those ephippial females that had eggs in their ovaries were used as this was the stage at which successful fertilization could occur.

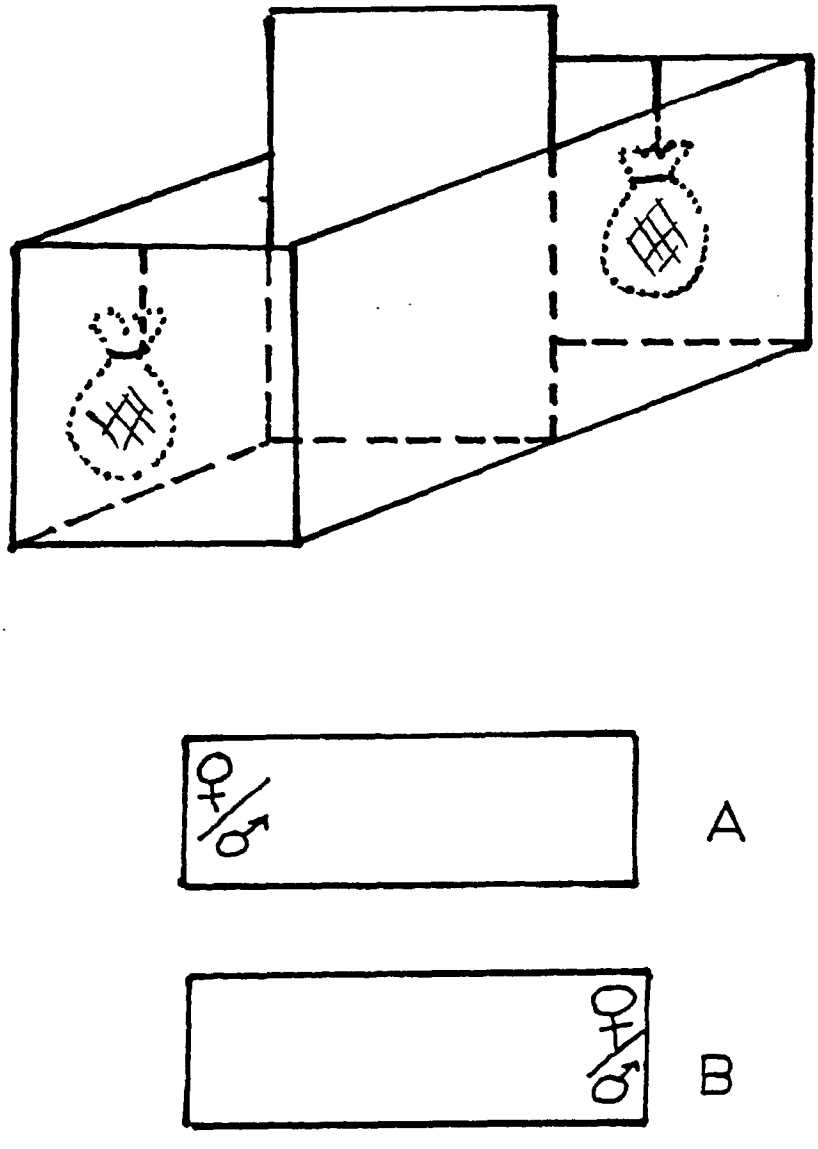
To begin an experiment, 10-30 males or sexual females were released in the center of the aquarium. After the animals appeared to be randomly distributed, 50-100 animals of the opposite sex were added to one of the two 164μ nitex bags located at either end of the aquarium. Every 30 minutes a plastic partition was placed in the center of the aquarium and the number of animals on each side was recorded. This process was repeated for 3½ to 4 hours.

Experiments were performed both with males free and

with females free. Two experiments (A and B) were run side by side with confined animals being placed at opposite ends of the two aquaria. Figure 7.1 diagrams the experimental apparatus.

Graphs of time versus proportion of animals on the side with the opposite sex were plotted for each experiment. Each observation within experiments was tested for concordance with a 1:1 ratio using X^2 analysis. If there was no attraction between the free and confined animals, one would expect the free animals to remain evenly distributed, that is, 50% on each side. The pattern of deviations from this ratio was examined. The experiments with females free were treated separately from those with males free.

Figure 7.1 Apparatus Used in Male-Female Attraction Experiments.



RESULTS

The results of the 14 individual experiments are shown in Tables 7.1 and 7.2. Six experiments were run with the females free and eight were run with males free. The animals were observed throughout the experiments and several interesting points were noted. Both males and females showed no preference for any particular area of the aquaria and were seen swimming near the surface as well as along the bottom. Individuals were often observed travelling the entire length of the aquaria only to turn around and start swimming back the other way. This suggested that individuals were likely to come in close contact with the confined animals during a trial. Furthermore, individuals that were seen near the bag of confined animals often swam away from it moments later. No swarms were observed around the confined animals. Swarming was seen only during the Nov. 30A trial. At 1 and 1.5 hours (Table 7.1) there were 17 females on the side with the males. These females were clustered in one corner of the aquarium while the bag of males was in the center of the water. To determine if this swarm was a result of the males' presence, the water in the aquarium was vigorously stirred. Table 7.1 snows that the females remained dispersed for the remainder of the experiment. The initial swarm was perhaps caused by an accumulation of food in the corner. If so, the swirling of the water redistributed the food so that the females were no longer at-

tracted to that spot.

X^2 values for each observation are shown in column four of Tables 7.1 and 7.2. The '+' and '-' signs in column five indicate where the animals were clustered when the distribution was significantly different from a 1:1 ratio (alpha = 0.05). A '+' sign indicates that the majority of free animals were on the side with the confined animlas while a '-' sign shows that they were on the other side. (The distribution of animals at time zero was not included in the analysis).

In both sets of experiments there were significantly more deviations from the 1:1 ratio than would have been expected on the basis of chance alone. In the "females-free" trials, seven deviations were found in a total of 38 observations. At the 5% level we would have expected to see two such deviations. Five of these were in favor of the confined animals but two were in the opposite direction. Most of the deviations occurred randomly throughout the six experiments except in the case of the Nov.30A trial which was discussed earlier.

In the "males-free" experiments, eight deviations were seen in a total of 50 observations where we would have expected to see two or three by chance. In all but one case the males were clustered on the side that did not contain the confined sexual females. Again, these deviations appeared to be randomly distributed among experiments. In three experiments the animals remained randomly distributed

Table 7.1 Results of Experiments With Females(F) Free and Males Confined.

Trial	Time hours	Count F	Count -	X^2	Direction of Deviation
1	0.0	9	11	0.20	
	1.0	13	7	1.80	
Nov.6A	1.5	14	6	3.20	
	2.0	9	11	0.20	
	2.5	11	9	0.20	
	3.0	9	11	0.20	
	3.5	9	11	0.20	
2	0.0	11	9	0.20	
	0.5	10	10	0.00	
Nov.6B	1.0	16	4	7.20	+
	1.5	12	8	0.80	
	2.0	12	8	0.80	
	2.5	10	10	0.00	
	3.0	13	7	1.80	
	3.5	12	8	0.80	
3	0.0	9	11	0.20	
	0.5	12	8	0.80	
Nov.30A	1.0	17	3	9.80	+
	1.5	17	3	9.80	+
	2.0	10	10	0.00	
	2.5	15	5	5.00	+
	3.5	12	8	0.80	
4	0.0	8	12	0.80	
	0.5	10	10	0.00	
Nov.30A	1.0	10	10	0.00	
	1.5	5	15	5.00	-
	2.0	10	10	0.00	
	2.5	11	9	0.20	
	3.0	9	11	0.20	
	3.5	7	13	1.80	
5	0.0	10	20	3.33	
	1.0	17	13	0.53	
Jan.29A	1.5	18	12	1.20	
	2.0	24	6	10.80	+
	2.5	13	17	0.53	
	3.0	12	18	1.20	
	3.5	10	20	3.33	

Table 7.1 Continued

Trial	Time hours	Count M	Count -	X^2	Direction of Deviation
6	0.0	14	16	0.13	
	1.0	9	21	6.53	-
Jan.29B	1.5	11	19	2.13	
	2.0	14	16	0.13	
	3.0	18	12	1.20	
	3.5	16	14	0.13	

Table 7.2 Results of Experiments With Males (M) Free and Females Confined.

Trial	Time hours	Count M	Count -	X^2	Direction of Deviation
7	0.0	8	12	0.08	
	0.5	4	16	7.20	-
Oct.18A	1.0	6	14	3.20	
	1.5	4	16	7.20	-
	2.0	6	14	3.20	
	2.5	8	12	0.80	
	3.0	7	13	1.80	
8	0.0	8	12	0.80	
	0.5	7	13	1.80	
Oct.18B	1.0	6	14	3.20	
	1.5	8	12	0.80	
	2.5	7	13	1.80	
	3.0	12	8	0.80	
	3.5	3	17	9.80	-
	4.0	7	13	1.80	
9	0.0	9	7	0.25	
	0.5	10	6	1.00	
Nov.23A	1.0	4	12	4.00	-
	1.5	9	7	0.25	
	2.0	11	5	2.25	
	2.5	9	7	0.25	
	3.0	11	5	2.25	
	3.5	3	13	6.25	-
	4.0	12	4	4.00	+
10	0.0	9	7	0.25	
	0.5	6	10	1.00	
Nov.23B	1.0	6	10	1.00	
	1.5	6	10	1.00	
	2.5	11	5	2.25	
	3.0	8	8	0.00	
	3.5	6	10	1.00	
	4.0	8	8	0.00	
11	0.0	4	6	0.04	
	1.0	7	3	1.60	
Jan.23A	2.0	5	5	0.00	
	3.0	5	5	0.00	
	4.0	4	6	0.40	

Table 7.2 Continued

Trial	Time hours	Count M	Count -	X^2	Direction of Deviation
12	0.0	6	6	0.00	
	1.0	8	4	1.33	
Jan.23B	2.0	7	5	0.33	
	3.0	7	5	0.33	
	4.0	5	7	0.33	
13	0.0	13	7	1.80	
	1.0	9	11	0.20	
Feb.13A	1.5	9	11	0.20	
	2.0	12	8	0.80	
	2.5	12	8	0.80	
	3.0	9	11	0.20	
	3.5	5	15	5.00	-
	4.0	8	12	0.80	
14	0.0	5	15	5.00	(-)
	1.0	8	12	0.80	
Feb.13B	1.5	8	12	0.80	
	2.0	10	10	0.00	
	2.5	5	15	5.00	-
	3.0	12	8	0.80	
	3.5	11	9	0.20	
	4.0	8	12	0.80	

throughout the entire trial while in the Nov. 23A experiment they were seen grouped on both sides at one time or another.

Although the non-random distribution of free animals on several occasions suggests that the environments within the aquaria were not entirely homogeneous, there is no evidence that the confined animals were the cause of this heterogeneity.

The results of the experiments are represented graphically in Figures 7.2 (a-f) and 7.3 (a-h). The horizontal line indicates the point at which half the animals are on each side of the aquarium. The graphs clearly show that there was no tendency for males to collect on the side with confined females, or for females to collect on the side with confined males.

Figure 7.2 Graph of Time vs Proportion of Free Animals on the Side With the Opposite Sex. Experiments With Females Free

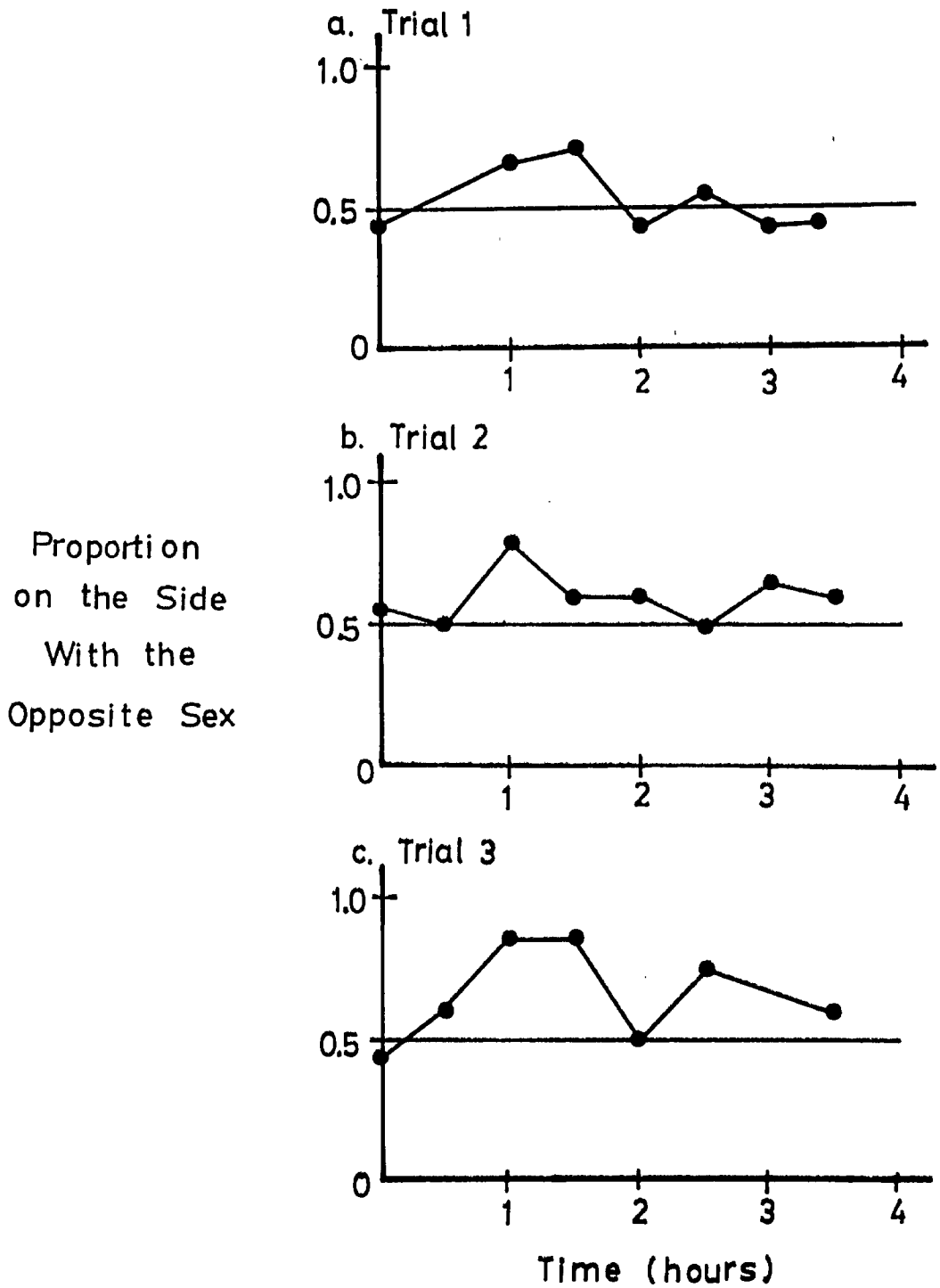


Figure 7.2 Continued

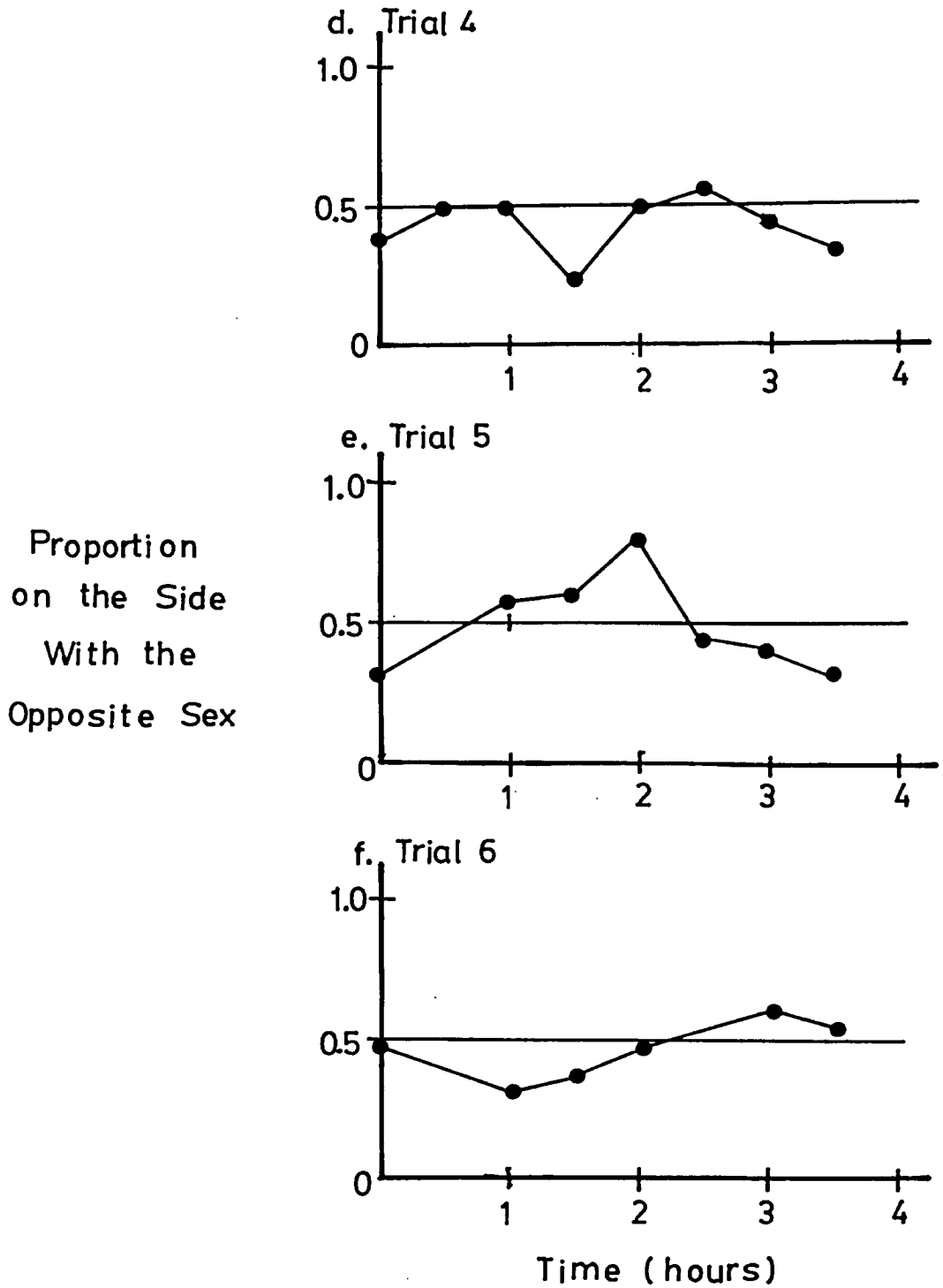


Figure 7.3 Graph of Time vs Proportion of Free Animals on the Side With the Opposite Sex Experiments With Males Free

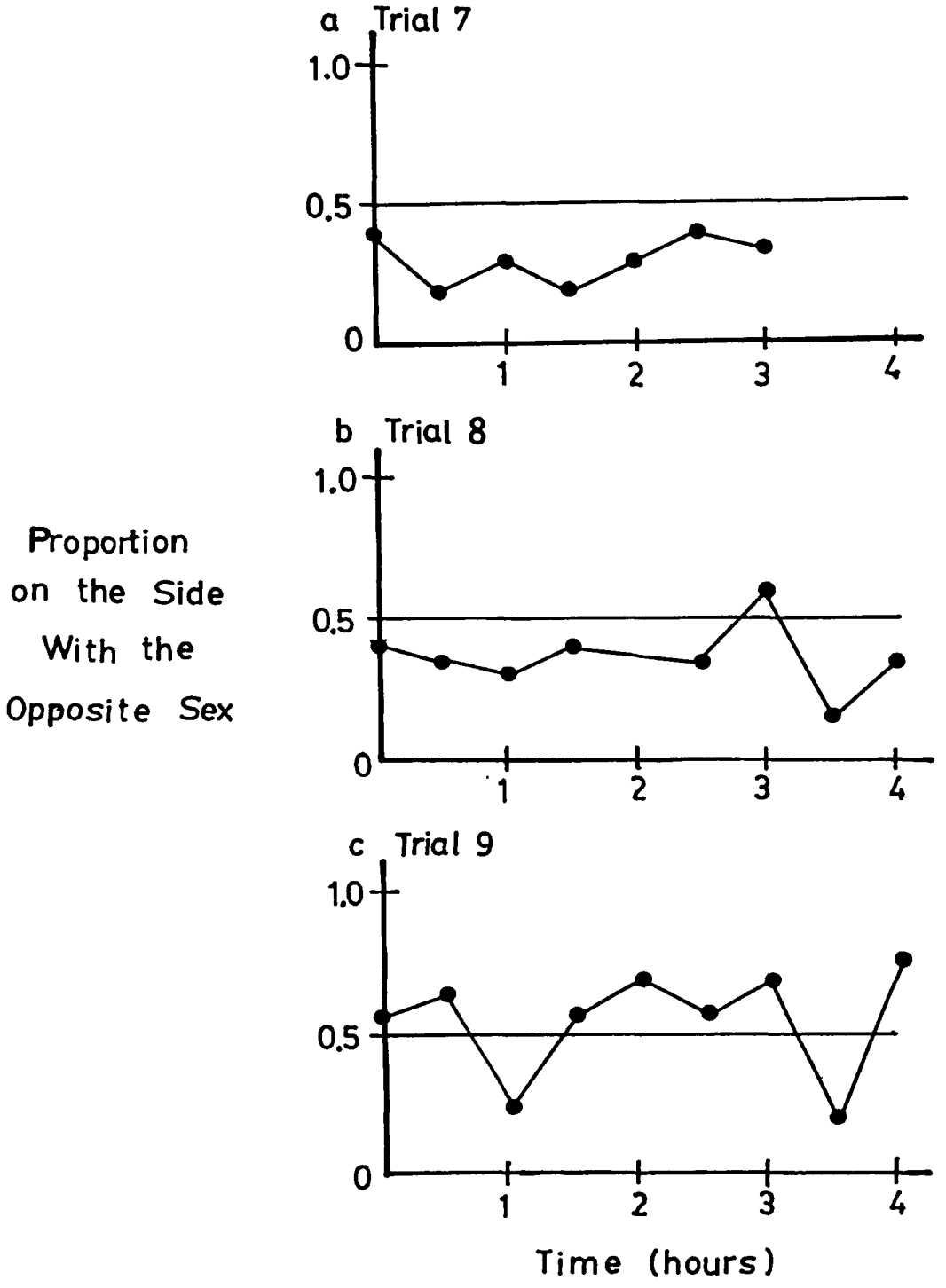


Figure 7.3 Continued

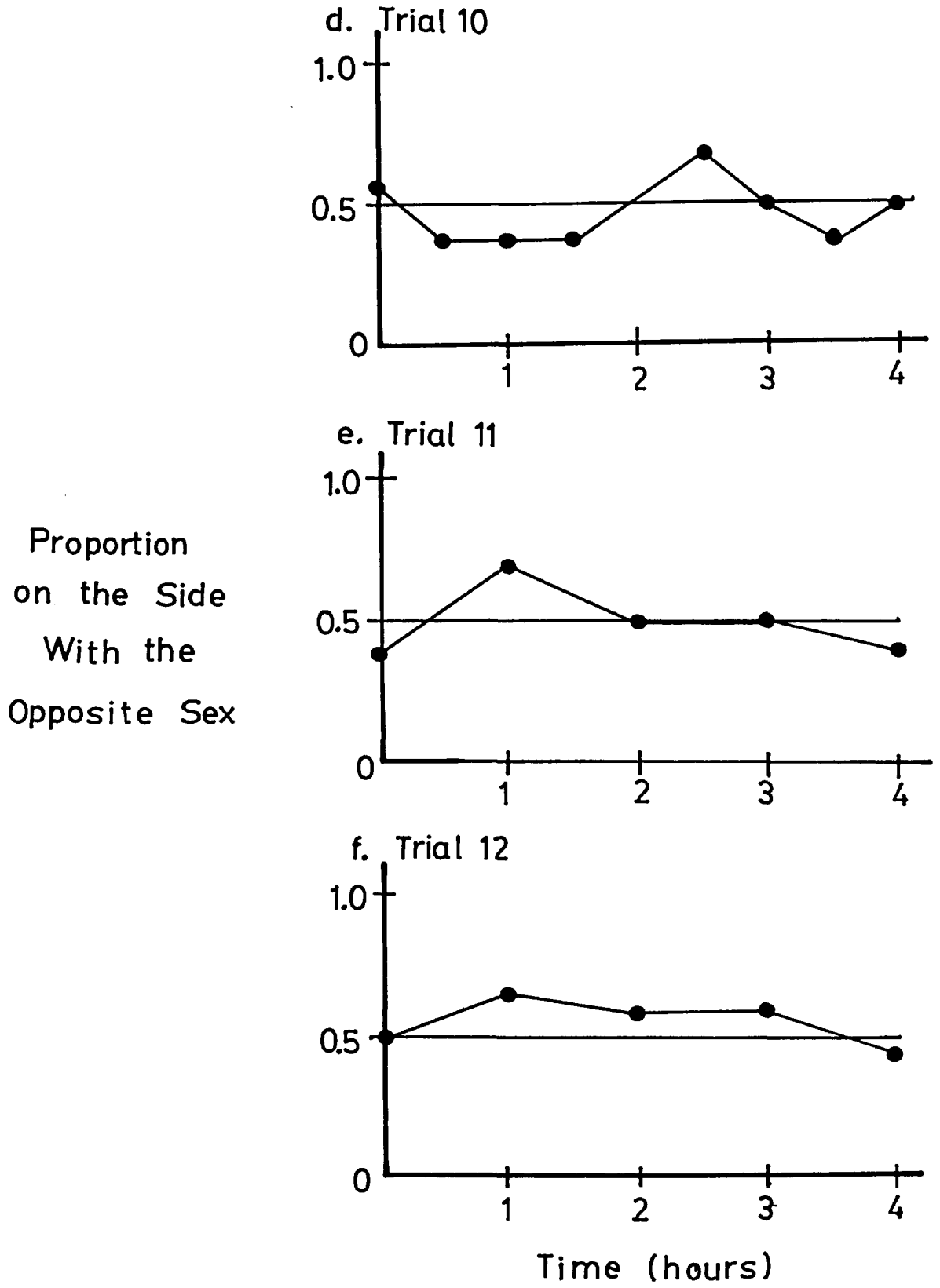
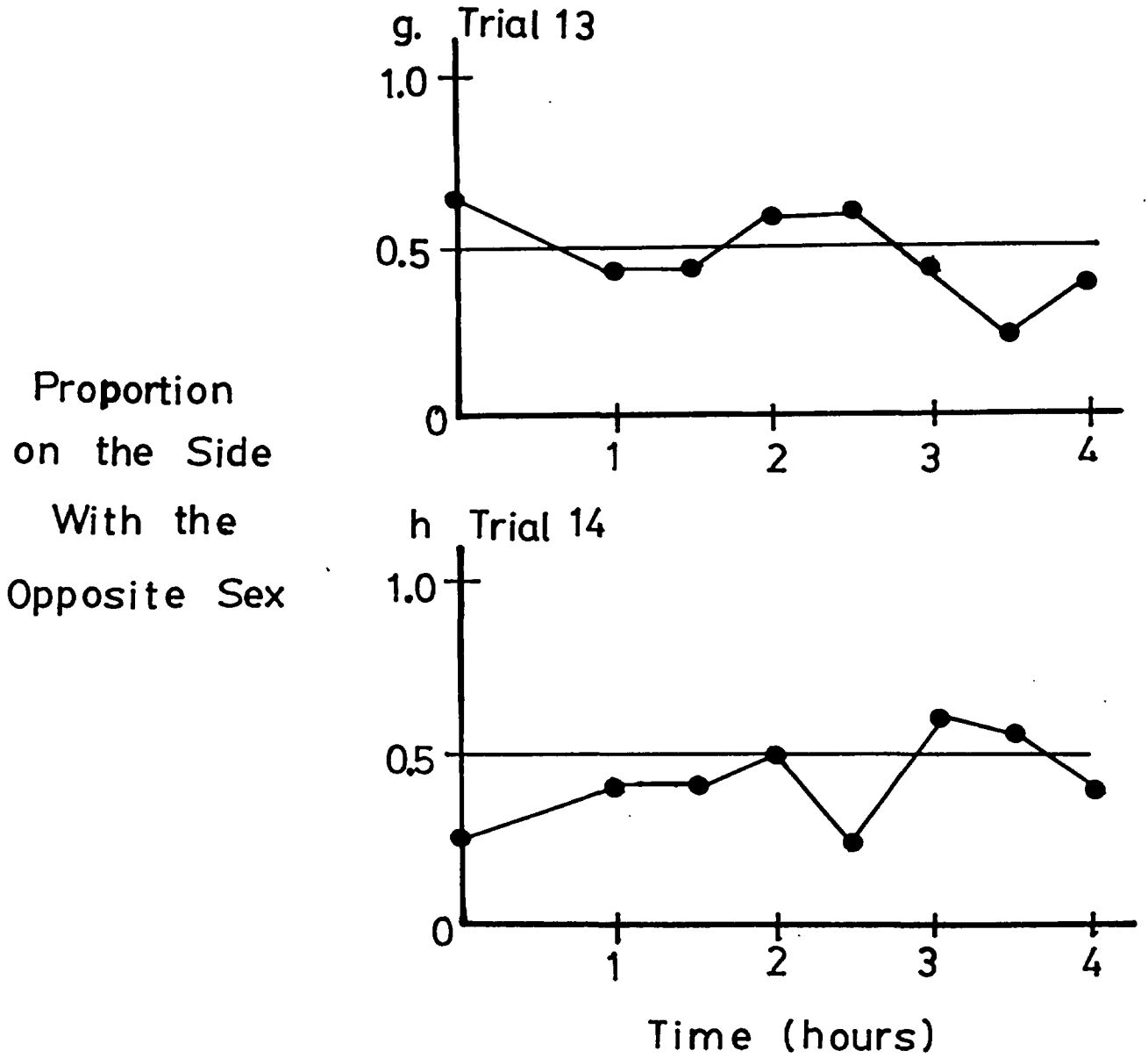


Figure 7.3 Continued



DISCUSSION

The results of these experiments provide no evidence of a chemically mediated attraction between male and female Daphnia magna. Neither sex showed any response to the presence of the opposite sex inside the nitex bags. Even animals that came in contact with the bags soon swam away. The concentration of sex phermone or similar chemical attractant, if present, should have been very high as there were 50-100 sexual individuals in each bag. Furthermore, the animals could be seen jostling about inside the bag so that any phermone released would certainly have been carried out into the surrounding water fairly quickly.

There have been several studies that do indicate the presence of phermones in some aquatic Crustaceans. Males of the marine crab, Portunus sanguinolentus mate with young females just after they molt. A male, upon finding a premolt female, carries her on his back until she is ready to mate. If such a female is placed in an aquarium with males they will begin display and search behaviour normally seen prior to mating. The same behaviour is observed in isolated males if water from an aquarium containing premolt females is added to the males' water. Intermolt and juvenile females do not elicit this response (Ryan, 1966).

Another study by Dahl et al. (1970) with the amphipod Gammarus duebeni showed similar results. If an aquarium

was divided into two sections using a nylon net, with males on one side and females on the other, the males would become very agitated and then swim towards the net and cling to it. If males were put on both sides, no response was observed. These examples show that rather dramatic changes in behaviour are associated with the presence of sex attractants. In contrast, the results obtained from this study of D. magna showed that no change in behaviour occurred in either sex when it was exposed to the other sex.

Gilbert (1963) found that rotifers of the genus Brachionus require contact between male and female to stimulate mating. When a male swims into a female, chemoreceptors on his corona receive an unknown chemical substance from her. Males only respond to this chemical if they perceive a sudden sharp increase in its concentration. In nature this can only be accomplished by direct contact between the sexes. Male Daphnia seem less discriminating as they often mate not only with parthenogenetic and nonreproductive females but also other males. These observations indicate that the presence of sexual females is not necessary to stimulate mating behaviour.

It seems likely that the Daphnia swarms described by Young (1978) were the result of an attraction to something other than phermones. For example, on one occasion Young dispersed a dense swarm by stirring it up yet a new one of the same size and density formed at approximately the same spot in about five minutes. If the Daphnia were attracting

one another by setting up some sort of phermone gradient in the water, the stirring should have dispersed the phermone and disrupted the gradient.

Young reported that the swarms he saw were confined to one shallow corner of the pond. Perhaps the Daphnia were attracted by the light intensity at that spot which may have been greater due to the shallowness of the water. It has long been established that Daphnia show a strong phototactic response (Clarke, 1930; Baylor and Hazen, 1962; Baylor and Smith, 1953). If I covered the top of an aquarium but left a 1" gap at one end (recall that the sides were covered with black paper) all the Daphnia in the aquarium moved towards the light very quickly. If the gap was suddenly moved to the other end, they all swam rapidly towards that side. All the animals would crowd together in the light space but when the cover was removed the group quickly dispersed and spread throughout the entire aquarium. If this was done when both males and females were present, the males usually arrived in the light area first.

There are other possible explanations for the Daphnia swarms observed by Young, for example they may have been attracted to an aggregation of algae. The important point is that given the results of my experiments and the behaviour of these swarms, there is no reason to suggest that male Daphnia magna attract female D. magna or vice versa. This seem to be a reasonable conclusion when one considers the circumstances under which sexual reproduction occurs.

Males are usually produced as a result of crowded conditions. Females produce ephippia in response to decreased food and generally deteriorating environmental conditions. As a result, sexual reproduction usually occurs during periods of high population density so that most individuals have an excellent chance of finding a mate. There is, therefore, little need for one sex to attract the other.

The reason for the association between sexual individuals and swarms remains unclear. The presence of males in the swarms may be related to their high mobility and their rapid response to attractive gradients. A swarm induced by physical conditions may itself be the stimulus for ephippial egg production by causing areas of high population density and local depletion of food resources.

Zooplankton ecologists have long recognized the patchy distribution of zooplankton populations. There is no reason to believe then that local concentrations of zooplankton occur only during periods of sexual reproduction. It is noteworthy that the correlation suggested in previous reports between swarming and sex probably results because sex occurs in times of extremely high population density. Swarming probably occurs under a variety of conditions but is most noticeable when population density is high. A change in density from 10 to 10,000 individuals per liter would be recognized immediately as a swarm but a change from 0.1 to 10 individuals per liter, although of the same order of magnitude, would hardly be noticed. Even though

swarming may, and probably does, occur during periods of low density it has not been recognized as such.

SUMMARY

Several zooplankton biologists have noted that swarming in Cladocera is often associated with periods of sexual reproduction. This has led one worker studying D. magna (Young, 1978) to suggest that members of one sex are able to attract members of the other sex. The most likely mechanism by which this could occur is through the production of phermones. As a result, experiments designed to determine if D. magna produced sex attractants were undertaken.

The results of these experiments provided no evidence for the presence of sexual phermones in D. magna. Both sexes were unaffected by the presence of the opposite sex in the nitex bags. It was suggested that the relationship between sexual reproduction and swarming may be due to the fact that sex only occurs during periods of high population density. It is during such periods that swarming behaviour would be most apparent. Swarming can most likely occur anytime but when population density is low, it is not recognized.

CHAPTER VIII

GENERAL DISCUSSION

CHAPTER VIII

The pattern of genetic variation seen in populations of Daphnia magna is quite different from that of other purely sexual invertebrates that have been studied. The level of variation in individual populations is somewhat lower than that seen in most other invertebrates (see General Introduction). Hebert (1972) has shown this to be due to inbreeding and founder effects in the English populations. Similarly it was suggested that the apparent lack of allozyme variation within Arctic populations was due to extreme founder effects. The area around Hudson Bay where Churchill is located was covered by the sea for a considerable period of time after the glaciers receded in the last ice age. As a result this area was probably one of the last to be re-colonized. The discovery that automixis may be involved in the production of ephippial eggs may be an alternate explanation for the increased homozygosity of these populations. It would be of great interest to study populations from glacial refuges in both Europe and North America to determine how their levels of variation compare to the populations studied here.

From this study it seems that genetic variation in this species tends to be maintained in the form of inter-population differences. Hebert found the allelic arrays of English populations which were meters away from one

another to be quite different. This pattern is even more apparent when the English and Arctic gene pools are compared. Gene substitutions had occurred at one half of the 16 allozyme loci examined and the mean genetic similarity between English and Arctic populations was only 0.62. This value is of the same magnitude as those found for sibling species of Drosophila. Recall that sibling species show complete reproductive isolation and yet hybridization studies with D. magna have shown that individuals from the English and Arctic metapopulations were quite capable of outcrossing with one another to produce viable, and in some cases, superior offspring. This observation tends to support Hebert's finding that increased levels of heterozygosity improve fitness. In situations where Hardy-Weinberg deviations were noted there was usually an excess of heterozygotes. This was the case for all polymorphic loci studied. Similar results were found by Young as well (1979 a,b).

In light of this it would be worthwhile to determine the results of introducing new alleles into an Arctic population. The prediction is that the new variation would be maintained in the population even in the face of strong inbreeding.

The situation in D. pulex populations provided a striking contrast to the results with D. magna. This species, thought to be a cyclic parthenogen was found instead, to be an obligate parthenogen in all populations that were ex-

amined from southwestern Ontario. This also seems to be the case in populations from Churchill, Manitoba and Kingston, Ontario (Hebert, pers.comm.). In 1925 Banta reported a population from Long Island, New York that appeared to possess this mode of reproduction.

The populations of D. pulex in the present study were found to consist of one to seven genetically distinct clones. Mean genetic similarity between clones based on 11 enzyme loci was 0.87. Subsequent work in these clones on other enzymes such as alkaline phosphatase, esterase and leucine amino peptidase has revealed several distinct phenotypes which would decrease the similarity between some clones even further. This shows that considerable genetic variation exists within D. pulex populations but it is distributed among asexual clones between which gene exchange is not possible.

In general, D. pulex individuals seemed to be heterozygous at a higher proportion of their loci than individuals of D. magna. Heterozygosity per locus per individual in D. magna from England was 0.07 and nearly zero in the Arctic. Conversely, D. pulex clones had, on average, heterozygosities of 0.15.

This leads to the question of why cyclic parthenogens maintain sexual reproduction and conversely, why obligate parthenogens have abandoned it. The advantage of producing offspring parthenogenetically during favorable conditions (i.e. summer eggs) cannot be disputed. Clones can

produce very large numbers of individuals very rapidly. The main concern is how to produce the ephippial eggs which will face new, unpredictable environments, either through colonization, re-establishment of intermittent populations or sexual recruitment into permanent populations.

Hebert (1978) points out that cyclic parthenogenesis allows the generation of many new genotypes through recombination during each sexual generation. Those genotypes best suited to the environment will then be able to replicate themselves unchanged by parthenogenesis throughout the growing season. When the environment begins to deteriorate sexual reproduction occurs producing a new array of genotypes. During each life cycle (meiosis to meiosis) the clones best suited for the local environment will be selected. Hebert suggests, then, that sexuality is retained in Daphnia populations for its role in local adaptation. Maynard Smith (1978) has suggested that if populations produce an ecologically differentiated stage sexually (ephippia), when there is the possibility that it could be produced asexually (as in obligate parthenogens), there must be some short-term advantage to sex. Otherwise, asexual reproduction would take its place. The ability to create new clones within habitats, thus increasing local adaptation, may provide that advantage.

One disadvantage of cyclic parthenogenesis seems to be that high levels of inbreeding often result causing the loss of some variability and a decrease in individual het-

erozygosity. Obligate parthenogens are able to avoid this problem by suppressing meiosis. White (1970) has suggested that heterozygosity should accumulate in apomicts. The higher heterozygosity of D. pulex clones as compared to D. magna populations seems to support this idea. Hebert's work in D. magna has shown that heterosis is important in determining the relative fitness of different genotypes.

Another advantage of obligate parthenogenesis is that it eliminates the need to produce males. This would be most advantageous for species in environments with short growing seasons. D. middendorffiana, which is found in the Arctic, is a good example but Hebert (1978) points out that obligate parthenogens are also found in temperate (D. pulex) and tropical (D. cephalata) habitats.

The major disadvantage of obligate parthenogenesis is the difficulty of constructing new genotypes. When a favorable mutation occurs in one clone it cannot be passed on to others. In addition, if a particular clone is lost through stochastic processes it cannot be regenerated unless it is introduced from another habitat. The study of D. pulex populations suggests that a limited number of clones are found in each pond (less than ten). In contrast, Young (1979a) found 29 clones of D. magna in one permanent population from England. It is likely that sexual recruitment of clones allowed the number to remain so high.

Hebert has proposed a model (see Chapter III) for the spread of obligate parthenogenesis in Daphnia popula-

tions. A mutation arises in a clone of a cyclic parthenogen which causes that clone to produce its ephippial eggs apomictically. But, normal males are produced which are able to spread the mutation to other clones when they fertilize normal haploid ephippial eggs. In such a system obligate parthenogenesis would spread very quickly throughout a population. The fact that obligate parthenogens such as D. pulex and D. middendorffiana are still capable of producing males supports this model.

It is hard to imagine what type of selection pressure could prevent such a mutation from reaching fixation unless it was also associated with a severe reduction in viability in individuals possessing it. Indeed, one would expect that obligate parthenogenesis in the Cladocera should be more common than is presently thought, unless such mutations arise very rarely. It would be of great importance to critically examine the breeding systems of other species of Daphnia, and of Cladocerans in general, as cyclic parthenogenesis is said to be the predominant mode of reproduction in the Order.

Maynard Smith (1978) feels that populations which have completely abandoned sexual reproduction have a limited evolutionary future. Then, are all species that adopt obligate parthenogenesis doomed to eventual extinction? The success of Daphnia species reproducing by this method seems to contradict this idea. The conflict concerning

the predominance of sexual reproduction in animals is far from being resolved, but further study of organisms such as Daphnia which have a variety of breeding systems should help provide answers to at least some of the questions that remain.

Appendix I. Computer Program to Construct a Dendogram Showing the Genetic Relationship Among Speices Using Nei's Genetic Distance.

```
//CLUS JOB (U12000T1D3),HEBERT
// EXEC WATFIV
//GO.SYSIN DD *
$JOB WATFIV U12000T1D3 HEBERT
C      PROGRAM FOR THE DENDOGRAM
       DIMENSION D(60,60),DD(50,50),I(50,50),ID(50)
       READ(5,1) KASE
     1 FORMAT(12)
5000   READ(5,1) N
       NN=N-1
       DO 100 L=1,NN
       KN=L+1
       READ(5,2) (D(L,M),M=KN,N)
     2 FORMAT(8F10,8)
   100 CONTINUE
       DO 300 L=1,NN
       KN=L+1
       DO 200 M=KN,N
   200 DD(L,M)=D(L,M)
       I(L,1)=L
       ID(L)=1
   300 CONTINUE
       I(N,1)=N
      ID(N)=1
2000 WRITE(6,3)
    3 FORMAT(1H1,6X3HI 1,6X3HI 2,6X3HI 3, 6X3HI 4, 6X3HI 5,
     6X3HI 6,6X3HI 7,6X3HI 8,6X3HI 9,6X3HI10,6X3HI11,6X3HI12,
     6X3HI13,6X3HI14/)
       KN=1
       DO 400 M=2,N
       WRITE (6,4) M, (DD(L,M),L=1,KN)
    4 FORMAT(1H ,1HJ,I2,14F9.5)
       IF(KN.GT.13) GO TO 400
       KN=KN+1
```

```
400 CONTINUE
    IF(N.LT.16) GO T) 499
    KNN=15
   WRITE(6,11)
 11  FORMAT(//,6X3HI15,6X3HI16,6X3HI17,6X3HI18,6X3HI19,6X3
     HI20,6X3HI21,6X3HI22,6X3HI23,6X3HI24,6X3HI25,6X3HI26,
     6X3HI27,6X3HI28/)
     DO 450 M=16,N
     WRITE(6,4) M,(DD(L,M),L=15,KNN)
 450 KNN=KNN+1
     IF(N.LT.30) GO TO 499
     KNNN=29
     WRITE(6,12)
  12 FORMAT(//,6X3HI29,6X3HI30,6X3HI31,6X3HI32,6X3HI33,6X3
     HI34,6X3HI35,6X3HI36,6X3HI37,6X3HI38,6X3HI39,6X3HI40,
     6X3HI41,6X3HI42/)
     DO 460 M=30,N
     WRITE(6,4) M,(DD(L,M),L=29,KNNN)
 460 KNNN=KNNN+1
 499 WRITE(6,5)
   5 FORMAT(///,10X23HORIGINIAL POPULATION NO./)
     DO 500 L=1,NN
     KNI=ID(L)
     WRITE (6,6) L,(I(L,K1),K1=1,KNI)
   6 FORMAT(1H ,5X1HI,I2,1H=,40I3)
 500 CONTINUE
     WRITE(6,5)
     DO 550 M=2,N
     KNJ=ID(M)
     WRITE(6,7) M,(I(M,K1),K1=1,KNJ)
   7 FORMAT(1H ,5X1HJ,I2,1H=,40I3)
 550 CONTINUE
     PREV=1000.0
     DO 700 L=1,NN
     KN=L+1
     DO 600 M=KN,N
     IF(DD(L,M).GT.PREV) GO TO 600
     PREV=DD(L,M)
```

```
      MI=L
      MJ=M
  600 CONTINUE
  700 CONTINUE
      WRITE(6,8) PREV
    8 FORMAT(///,10X19HMINIMUM DISTANCE IS,F9.5)
      KNI=ID(MI)
      WRITE(6,9) (I(MI,K1),K1=1,KNI)
    9 FORMAT(1H ,3X7HBETWEEN,40I3)
      KNJ=ID(MJ)
      WRITE(6,10) (I(MJ,K2),K2=1,KNJ)
   10 FORMAT(1H ,3X3HAND,40I3)
      DO 1200 L=1,N
      IF(L.GT.MJ) GO TO 800
      IF(L.EQ.MI) GO TO 1000
      GO TO 1200
  800 KN=ID(L)
      DO 900 K1=1,KN
  900 I(L-1,K1)=I(L,K1)
      ID(L-1)=ID(L)
      GO TO 1200
 1000 KN=ID(MJ)
      DO 1100 K1=1,KN
      K2=ID(L)+K1
 1100 I(L,K2)=I(MJ,K1)
      ID(L)=ID(L)+KN
 1200 CONTINUE
      DO 1900 L=1,NN
      KN=L+1
      IF(L.GT.MJ) GO TO 1700
      IF(L.EQ.MJ) GO TO 1900
      IF(L.EQ.MI) GO TO 1850
      DO 1600 M=KN,N
      IF(M.GT.MJ) GO TO 1300
      IF(M.EQ.MI) GO TO 1400
      GO TO 1600
 1300 DD(L,M-1)=DD(L,M)
```

```
      GO TO 1600
1400  AK=0.0
      SUMD=0.0
      NI=ID(L)
      NJ=ID(M)
      DO 1500 L1=1,NI
      DO 1500 M1=1,NJ
      L2=I(L,L1)
      M2=I(M,M1)
      IF(L2.GT.M2) GO TO 1450
      SUMD=SUMD+D(L2.M2)
      GO TO 1500
1450  SUMD=SUMD+D(M2,L2)
1500  AK=AK+1.0
      DD(L,M)=SUMD/AK
1600  CONTINUE
      GO TO 1900
1700  DO 1800 M-KN,N
1800  DD(L-1,M-1)=DD(L,M)
      GO TO 1900
1850  NI=ID(L)
      DO 1860 M=KN,N
      AK=0.0
      SUMD=0.0
      NJ=ID(M)
      DO 1870 L1=1,NI
      DO 1870 M1=1,NJ
      L2=I(L,L1)
      M2=I(M,M1)
      IF(L2.GT.M2) GO TO 1855
      SUMD=SUMD+D(L2,M2)
      GO TO 1870
1855  SUMD=SUMD+D(M2,L2)
1870  AK=AK+1.0
      DD(L,M)=SUMD/AK
1860  CONTINUE
```

```
 1900 CONTINUE
      N=N-1
      NN=N-1
      IF(N.GT.1) GO TO 2000
      KASE =KASE-1
      IF(KASE.GT.0) GO TO 5000
 5000 RETURN
      STOP
      END
$ENTRY
```

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